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81

Problems of D
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Proceedings of the
49th Annual Scientific Meeting

The Committee on Problems
of Drug Dependence, Inc.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES • Public Health Service • Alcohol, Drug Abuse, and Mental Health Administration

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Problems of Drug Dependence, 1987

Proceedings of the 49th Annual Scientific Meeting, The Committee on Problems of Drug Dependence, Inc.

Editor: Louis S. Harris, Ph.D.

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NIDA Research Monographs are prepared by the research divisions of the National Institute on Drug Abuse and published by its Office of Science. The primary objective of the series is to provide critical reviews of research problem areas and techniques, the content of state-of-the-art conferences, and integrative research reviews. Its dual publication emphasis is rapid and targeted dissemination to the scientific and professional community.

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Foreword

The National Institute on Drug Abuse (NIDA) is pleased once again to publish in its Research Monograph series the proceedings of the 49th Annual Scientific Meeting of the Committee on Problems of Drug Dependence, Inc. (CPDD). This meeting was held in Philadelphia, Pennsylvania, in June 1987 and was cosponsored by the Research Society on Alcoholism. Consequently, it was unusually well attended, and the meeting resulted in the largest number of papers and abstracts in the history of CPDD.

The CPDD is an independent organization of internationally recognized experts in a variety of disciplines related to drug addiction. They are interested in developing knowledge that will reduce the destructive effects of abused drugs on the individual and society. The CPDD maintains liaison with Government regulatory and research agencies, with the pharmaceutical industry, with national and international organizations interested in drug dependence and abuse, and with those working on drug abuse problems in academic and treatment settings. The Committee is unique in bringing together annually at a single scientific meeting an outstanding group of basic and clinical investigators working in the field of drug dependence. This year, as usual, the monograph presents an excellent collection of papers. It also contains progress reports of the abuse liability testing program funded by NIDA.

The contents of this monograph comprise a "state-of-the-art" summary of ongoing research into the biological, behavioral, and chemical bases of drug abuse that should be valuable to readers with a wide diversity of interests.

Khursheed Asghar, Ph.D.
Division of Preclinical Research
National Institute on Drug Abuse

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The Committee on Problems of Drug Dependence, Inc., an independent, nonprofit organization, conducts drug testing and evaluations for academic institutions, government, and industry. This monograph is based upon papers or poster presentations from the 49th Annual Scientific Meeting of the CPDD, held in Philadelphia June 14 - 19, 1987. In the interest of rapid dissemination, it is published by the National Institute on Drug Abuse in its Research Monograph series as reviewed and submitted by the CPDD. Dr. Louis S. Harris, editor of the monograph, is Chairman of the Department of Pharmacology, Medical College of Virginia. During 1987 Dr. Harris also served as Senior Science Advisor to the Director of NIDA.

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Initiatives at the National Institute on Drug Abuse

C. Schuster

It is a pleasure and an honor to be here with you today. I bring greetings from Dr. Macdonald in his dual role as Administrator of the Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA), as well as Director of the White House Drug Abuse Policy Office. Right now, he is working closely with the First Lady on preparations for the International Conference on Drug Abuse and Illicit Trafficking, and I will be leaving from here to take part with them in that meeting in Vienna.

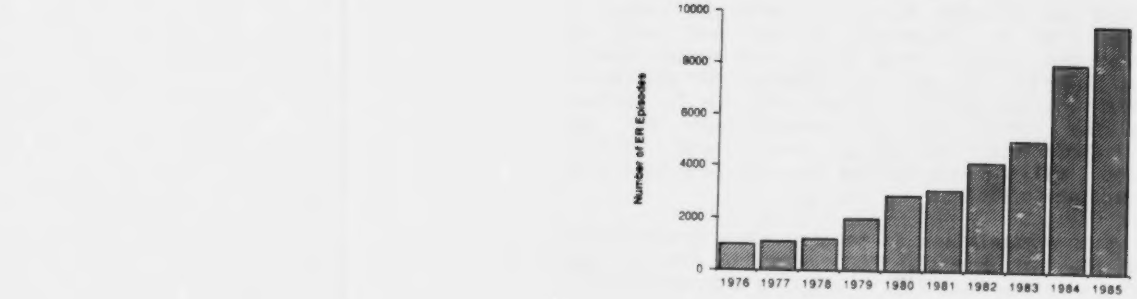
My purpose in this presentation is to give you a brief status report on drug abuse in general, NIDA's research efforts, and other programmatic issues that NIDA is dealing with currently.

As you know, the prevalence rates for most illicit drug use peaked in 1979-80 and have declined since then. Cocaine is the exception to the overall pattern. Its use has shown a small but significant increase over the same period of time. It is important to note that, even with cocaine, the large increase in usage occurred between 1977 and 1980, and the subsequent rise in usage has been much slower.

Despite this overall slowing in the use of illicit drugs, there has been unprecedented public interest and concern with this subject in the past year. For example, in a survey conducted by NIDA in August, 1986, fully 73 percent of a random sample of 1,001 Americans age 18 and older said that illegal drug use is "one of the most serious problems facing the country." Twenty-five percent said that drug use is "an important problem for the country." Only two percent did not consider it an important problem.

I believe that this interest and concern stems from two phenomena. First, there has been a great increase in both the incidence and the visibility of cocaine-associated public health problems. NIDA's data show alarming increases in cocaine-related emergency room admissions, medical examiner reports, and demand for treatment (Figure 1). Second, people are becoming more and more aware that AIDS is spreading from needle-sharing among intravenous drug users and, from them through sexual contacts, to the general heterosexual population.

On the other hand, I won't hesitate to point out that "fame and fortune" can be fleeting in Washington, D. C. I was told only last week by a Congressional staff person that drug abuse is last year's issue, and Congress is focussing more on the emerging issue of homelessness. This illustrates a basic problem we have in generating sustained interest in the support of drug abuse research, prevention, and treatment.



NUMBER OF COCAINE-RELATED ER EPISODES IN TOTAL CONSISTENTLY-REPORTING PANEL: 1976-1985

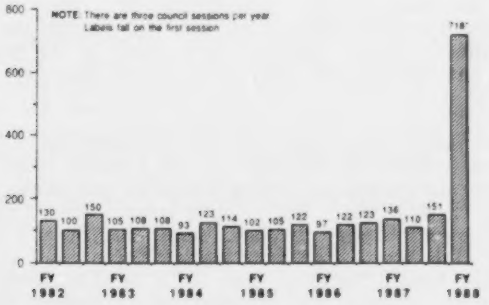
It is up to us, who have long-term interest in the field of drug abuse, to carry the message that drug abuse is not a problem that can be solved and forgotten. Rather, just as we consider heroin addiction to be a chronic disorder leaving individuals subject to relapse, we must regard drug abuse as a chronic disorder afflicting society, which is also vulnerable to relapse. We all know that the prevalence of drug abuse waxes and wanes. In my opinion, this vacillation occurs because of a waxing and waning of activities to control drug abuse. When prevalence rates are driven down by societal interventions, there is a relaxation of control measures and the low-level endemic problem spreads. A parallel situation can be seen when vaccinations reduce the rate of a particular disease, everyone sighs with relief, people forget to have their children vaccinated, and the disease rears its ugly head again. When we succeed in reducing drug abuse, we turn our attention to other concerns until the prevalence rates climb back to a point at which society becomes concerned enough to react. There is then a call for research to develop prevention and treatment strategies to solve the problem.

I would like to believe that we can do better than that in the future. But this will demand a steady attention to the problem of drug abuse, even when it is at a level that is not "bothering" the general public. Only when we understand the complex biobehavioral processes responsible for addictions will we be in a position to develop maximally effective prevention and treatment interventions. Sustained support of research and demonstration grants is essential if we are to reach this goal.

Let me turn now to the current grant situation at NIDA. It looks as though NIDA will have enough excellent grant applications to permit a useful allocation of all available research funds for FY87. As of April 1, which was a special deadline for application, we had received more than 800 grant proposals (Figure 2). This is an extraordinary increase, and includes applications from previous grantees as well as researchers who are new to the drug abuse field or, at least, new to NIDA. Twenty-two center grant applications or supplements were submitted, as opposed to the usual two or three. Let me note that at this time, of course, we still don't know how much of the $26 million authorized for research will be used for multi-year funding or whether it all can be allocated to new starts. But in any case, there is plenty of material to review, and we are fortunate to have the assistance of Dr. Louis Harris, who is coordinating the review process.

NIDA PROJECT GRANT HISTORY

NUMBER OF NEW AND COMPETING PROJECT GRANT APPLICATIONS



* Excludes training grants, fellowships, and Small Business Innovative Research grants

I want to move on to mention some areas of special priority for NIDA and, indeed, for ADAMHA. The first is prevention. We are concerned with populations that are at high risk of drug abuse, whether for genetic, environmental, or social reasons. The new Office of Substance Abuse Prevention in ADAMHA is offering demonstration grant money to address the problems of certain high risk populations. NIDA is conducting basic research to identify such populations and to develop and evaluate interventions that will prevent addiction. In light of the AIDS epidemic, special efforts are aimed toward prevention and early intervention in geographic areas in which intravenous drug abuse is prevalent.

Treatment research is another area of great interest to NIDA. I want to stress that treatment research, in this context, includes all pre-clinical research which will have relevance to the development of new treatment approaches. It is not limited to purely clinical research. NIDA's intramural and extramural programs are involved in a broad variety of treatment research projects. For example, the Addiction Research Center (ARC) is evaluating current treatments for cocaine dependence. Treatment programs across the country are utilizing a variety of behavioral and pharmacologic approaches for the treatment of cocaine addiction, but there is little hard evidence demonstrating their efficacy. Another focus of NIDA is toward the development of new pharmacologic and behavioral treatment modalities for all forms of drug dependence, but with special emphasis placed on cocaine. Anti-depressants are showing promise in tests of their ability to lessen cocaine cravings. Again because of AIDS, we are also emphasizing the treatment of heroin addiction in an attempt to enlarge our armamentarium of approaches. Buprenorphine has been demonstrated to block the reinforcing effects of opiate in rhesus monkeys and humans in controlled laboratory conditions. Studies at the ARC (and elsewhere) are now investigating its usefulness in heroin addicts seeking treatment.A spectrum of treatment methods should draw more addicts into treatment. To paraphrase Dr. Beny Primm, methadone and therapeutic communities are not enough--we need a "shopping center" where addicts can find a variety of approaches and can choose the ones with which they are most comfortable.

In its research, NIDA also looks at the consequences of drug abuse. Functional deficits related to drug abuse that have been noted in the workplace are being explored and measured in the laboratory. At the ARC, scientists are studying drug-induced neuropathology through some important animal experiments and with the aid of such modern techniques as PETT scanning. A NIDA-funded Maternal and Child Health Survey has brought forth some interesting information in regard to the teratogenic effects of cocaine.

Let me emphasize that these examples are only illustrative of our research priorities. They are by no means exhaustive.

Now I want to mention some programmatic issues NIDA has faced in the past year. There has been major development in two areas, AIDS and Workplace Initiatives.

The Office of Workplace Initiatives was established within NIDA in response to the President's call for a drug-free

American workplace. Its Director, Dr. Michael Walsh, will be establishing programs to deal with urine drug testing, employee assistance programs, and referrals for treatment (access to the system). NIDA has developed general guidelines for drug screening, as well as technical guidelines that the Federal government will follow in testing its own employees. In addition, the accreditation of laboratories that wish to perform urine drug testing is being addressed. We are determined to ensure that drug testing is performed accurately if it is to be done at all.

NIDA's efforts against AIDS are coordinated in the Division of Clinical Research (DCR), headed up by Dr. Roy Pickens. However, many parts of the Institute are involved in various anti-AIDS initiatives. Seroprevalence studies have been conducted by ARC staff. Ethnographic studies of needle sharing are the responsibility of the Division of Epidemiology and Statistical Analysis. The Office of Research Communications is handling the development of an educational campaign that will utilize mass media techniques. Some materials are now awaiting clearance. DCR expects to award demonstration grants that will assist five cities in organizing comprehensive outreach, intervention, and treatment programs. Also, targetted grants will help other cities through smaller but equally vital programs. In addition, a contract will be awarded to coordinate data collection from these programs and to provide some evaluation of their effectiveness.

NIDA is currently analyzing policy issues that have relevance to the AIDS epidemic. Among these are the scientific, legal, and ethical questions surrounding mandatory treatment, the utility and desirability of needle exchange programs; and the question of how drug treatment capacity can best be increased to meet the challenge posed by AIDS. These and other policy concerns will be reviewed on an ongoing basis.

In summary, we have the public's attention and its interest right now. How long this will continue is unpredictable. AIDS will keep the public interest high, at least for the next five years, but the problem of drug abuse is very broad and very deep. We have a lot of work left to do.

And now I must close and literally run to join a party of 32 delegates who are leaving on Air Force 1 this afternoon to attend the International Conference on Drug Abuse and Illicit Trafficking. This is the first ministerial level meeting in which both supply and demand reduction authorities will convene for developing integrated international strategies for decreasing the world-wide problem of drug abuse. I am very sorry that this meeting precludes my being here to participate in the remainder of this scientific meeting. Fortunately, the proceedings of this meeting will be published, as in the past, as a NIDA Monograph.

AUTHOR: Charles R. Schuster, Ph.D., Director
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Presentation of Nathan B. Eddy Memorial Award

W. Martin

I am very pleased to introduce the 1987 recipient of the Nathan B. Eddy Award. It is a long overdue recognition of Dr. C. K. Himmelsbach's contributions to drug dependence. I am pinch hitting for Dr. Harris Isbell who very much wanted to attend this meeting honoring his old friend and predecessor as Director of the Addiction Research Center. Dr. Isbell felt reluctant to make this trip and regrets his absence.

Dr. Himmelsbach was Director of the Addiction Research Center from 1935 to 1944. Following his tenure at the Addiction Research Center, he served at the National Institutes of Health as Assistant Director of the Clinical Center. Following his retirement from the Public Health Service, he became an Associate Dean at Georgetown University School of Medicine and Dentistry.

There are few in this audience that have not been the beneficiary of Dr. Himmelsbach's efforts and creative mind. Dr. Himmelsbach was a Public Health Service physician who was especially identified to become the Addiction Research Center's first Director. In preparation for this responsibility, he spent a yaer in the laboratory of Dr. Torald Sollmann where he worked on problems of morphine tolerance and dependence in rats. He then worked with Nathan B. Eddy at the University of Michigan where he continued his pharmacologic studies prior to initiating his first clinical studies of addiction at the Leavenworth penitentiary. When the United States Public Health Service Hospital (NARCO) opened in Lexington, Kentucky, in 1935, Dr. Himmelsbach continued his studies at this facility. He developed the abstinence scoring system and was the first to quantify the morphine abstinence syndrome in man. This scoring system has not only served a number of investigators as an insightful and practical method for measuring the intensity of abstinence, but also was a model for quantitatively studying withdrawal and precipitated abstinence. When Harris Isbell employed bioassay techniques to analyze suppression data, the great power of this instrument was fully demonstrated. He also devised the suppression technique for classifying morphine-like drugs. Although receptor theory had not, in 1935, become a part of pharmacologists' and physician's way of thinking about drug actions, the suppression technique was one of the first clues that a number of opioids were acting through a common mechanism. Dr. Himmelsbach compared the actions of a number of morphine-like analgesics. He also evaluated the effects of several popular treatments for the morphine abstinence syndrome.

Dr. Himmelsbach was the first to carefully characterize the duration of the morphine abstinence syndrome and recognize that it took many months for this process to resolve. Dr. Himmelsbach's homeostatic theory of tolerance and physical dependence, with the dual action theory of Tatum, Seevers and Collins, were the first attempts to provide a pharmacologic explanation of opioid dependence. The homeostatic theory still attracts interest and is a seminal theory. Dr. Himmelsbach's work has had a profound influence on my work, as well as on many others in this room, and I deem it a high honor to introduce the recipient of the Nathan B. Eddy Award in 1987, Dr. Clifton K. Himmelsbach.

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Clinical Studies of Morphine Addiction: Nathan B. Eddy Memorial Award Lecture

C. Himmelsbach

The last time I met with you was May, 1975, when I had the honor of presenting "An Historical Review of Research on Drug Addiction" at your Washington Meeting. Today, in this City of Brotherly Love, you do me an even greater honor by making me the recipient of the prestigious Nathan B. Eddy Award. I am truly grateful.

During the period of my activity in this field, I had the pleasure and benefit of a close association with Dr. Eddy. Our friendship continued throughout the remainder of his life-time. This gives added meaning to having his likeness on my new medal.

Also, it is highly significant that this event takes place in Philadelphia for I was born here 80 years ago; my parents are buried here; and my brother lives here. Although I have lived elsewhere most of my life I am especially glad to be here today.

Dr. Way suggested that you might be interested in a summary of my research with particular reference to:

quantification of the abstinence syndrome,
testing for addiction liability, and
the nature of physical dependence.

But first, I think it is important to emphasize that, except for the work of Light and Torrance (done right here in Philadelphia), clinical research on morphine addiction was a wide-open field when I started. I enthusiastically undertook to do the things I was trained to do and was fortunate to have the opportunity to contribute some base for the far-reaching studies now being conducted by investigators here present. We all know that success in research, as in most things, is dependent in some measure on luck. I had my share.

QUANTIFICATION OF THE MORPHINE ABSTINENCE SYNDROME

As a novice in this field, and in preparation for the opening of the Lexington Hospital, I first needed to gain familiarity with addiction in man. For this purpose I was assigned to the U.S. Penitentiary Annex in Ft. Leavenworth, Kansas were addicts then served their sentences for violations of the Harrison Narcotic Act. I was given a 12 bed ward, staffed with a nurse and three attendants, and a laboratory technician. My first experience was with a "chain" of 10 actively ill addicts from New York City. Since there were too many to cope with at one time, I gave them enough morphine to keep them comfortable, and then observed the events which took place following abrupt withdrawal of the drug from one patient at a time. It soon became clear that this was a quite uniform and predictable illness which ran its main course in about 10 days. Also, it lent itself to equating clinical judgement of its severity with the appearance of a pattern of signs which we first expressed as Mild (+), Moderate (++), Marked (+++), or Severe (++++). (Figure 1)

While this was an improvement over narrative descriptions, it did not distinguish between fine gradations of severity. So, adhering to Pythagoras' dictum that "numbers are the language of nature," arbitrary numeric values were given to the component signs. After several trials we settled on the system shown in the next (Figure 2) which gave a closer approximation to clinical judgement, was smoother than 1+, 2+, 3+ and 4+, and lent itself to graphic portrayal of this illness. (Figure 3)

It soon became apparent that there was a clear relationship between the intensity of the abstinence syndrome and dosage of morphine. (Figure 4)

TESTING FOR ADDICTION LIABILITY

Your predecessor committee had embarked on a scientific search for a non-addictive substitute for morphine. To this end, a Chemistry Laboratory had been established at the University of Virginia under Dr. Lyndon F. Small for the purpose of developing a host of variations of the morphine molecule, and synthesizing new potential analgesics. These were studied pharmacologically by Dr. Eddy at the University of Michigan to learn of relationships of pharmacological actions to chemical structure. Those which showed analgesic promise were to be tested for addiction liability in man. That was my job.

The substitution method for accomplishing this came about fortuitously when I attempted to replicate in man the demonstration of cross-tolerance to several opiates in dogs by Downs and Eddy. Various derivatives of morphine were substituted for the parent drug in addicts who were receiving enough morphine to prevent abstinence signs. Not only was cross-tolerance evident, but when the substituted drug was withheld about one week later, a full-blown abstinence syndrome appeared. (Figure 5) I seemed to have grafted one dependence on another. This led to the view that an agent which could support established dependence would probably produce it de novo.

Experience with many narcotics favored the validity of this assumption. Moreover, it seemed to apply in degree as well as kind. Thus, codeine ranked as weakly addictive, while Dilaudid was powerfully so. But, unfortunately, it did not seem possible to dissociate addiction liability and analgesic power. In fact, the two seemed to go together. Crucial to the validity of the substitution hypothesis was our trial of the then new synthetic analgesic "Demerol". Much to the surprise (and disappointment) of many, it tested positive and clinical experience has borne out its addiction liability.

While the substitution method was valuable, it was slow and cumbersome. In searching for a better way to detect addiction liability, we based our approach on the information gained from checking out many so-called "withdrawal treatments".

These, including tetrahydrocannabinol, had no detectable effects on the abstinence syndrome, whereas morphine had a pronounced effect. (Figure 6)

The morphine abstinence syndrome is so uniform and predictable, especially for the first 48 hours, that it lends itself to measuring the effects, if any, of a drug administered at the 30th hour. The effect of 10mg. morphine is shown in (Figure 7).

Neither normal saline nor thiamine had any effect when administered at the 30th hour, whereas codeine, morphine, morphine with prostigmin, morphine sulfuric ether, and demerol had significant and measurable effects. (Figure 8) This, then, seems to offer a quick and easy means for detecting addiction liability.

ON THE NATURE OF PHYSICAL DEPENDENCE

Another assigned task was to try to unravel the mystery of physical dependence. What was it that made morphine seem to become a biological requirement? What changes occurred as a result of chronic use? Were there biological changes that predisposed to relapse? Or was the strong tendency to recidivism entirely psychological and environmental?

Early on, I was impressed with the resemblance of the morphine abstinence syndrome to an intense and prolonged "tempest" occurring in the sympathetic division of the autonomic nervous system--perhaps even centered in the region of hypothalamus. (Figure 9) This impression grew stronger with continued observations and led to a rather simplistic explanation of physical dependence as a compensatory physiologic response to repetitive disturbances in homeostasis caused by morphine. Thus, it seemed possible that dependence could be an adaptive phenomenon resulting from periodic restoration of steady states upset by morphine. (Figure 10)

If one considers homeostasis to be achieved by balanced forces that "act" and "counteract" as required to preserve phylogenetically established levels of "normality", then repeated pharmacologic interference could tend to substitute and thereby weaken one and reciprocally strengthen the other--so that, in time, the interfering agent would become a pseudobiologic substitute and thus become a requirement for achieving a semblance of homeostasis. Inevitably, as the drug is metabolized the need arises for a new supply, and thus dependence manifests itself--or, in the addict's parlance, the user has become "hooked".

To seek greater insight on chronic effects, I carried out long-range follow-up observations and investigations directed at a better understanding of the role of the autonomic nervous system. As controls, we were fortunate to have access to marijuana users who had no history of taking opiates, and who were serving sentences under identical living conditions.

It became apparent that physical recovery was a very slow process with measurable effects of dependence still detectable up to six months after withdrawal. Even more significant were evidences of hyperactivity of the sympathetic division of the autonomic nervous system persisting up to two years in prisoners serving long sentences. Thus it would appear that recidivism may be abetted, in part, by what seem to be indelible effects of addiction on the nervous system.

At this point I wish to pay tribute to the many, many patients who made these studies possible. Despite the fact that they were incarcerated, they were volunteers in the strict sense of the word. While our studies predated the Nuremberg Trials, the guidelines they formulated for research on man were implicit in our patient-physician relationship. We obtained simple consent. (As to its being "informed", I am sure the patients knew much more about narcotic drug effects than we did). Patients were privileged to withdraw from the protocol at will, and we could terminate or modify the protocol as indicated. To the best of my knowledge none was harmed by the experience.

Unfortunately, however, we did not find a non-addictive substitute for morphine. Nor, despite the beautifully orchestrated rehabilitation program carried out at Lexington, were we able to make a significant impact on the relapse rate. But, I do hope that the basic studies summarized today have been helpful to your attack on this frightful problem. I wish you success and good luck!

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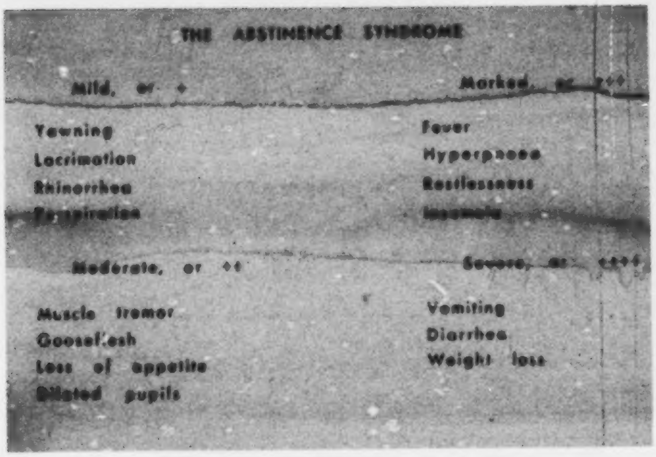


THE ABSTINENCE SYNDROME

Mild, or +

Yawning
Lacrimation
Rhinorrhea
Perspiration

Moderate, or ++

Muscle tremor
Gooseflesh
Loss of appetite
Dilated pupils

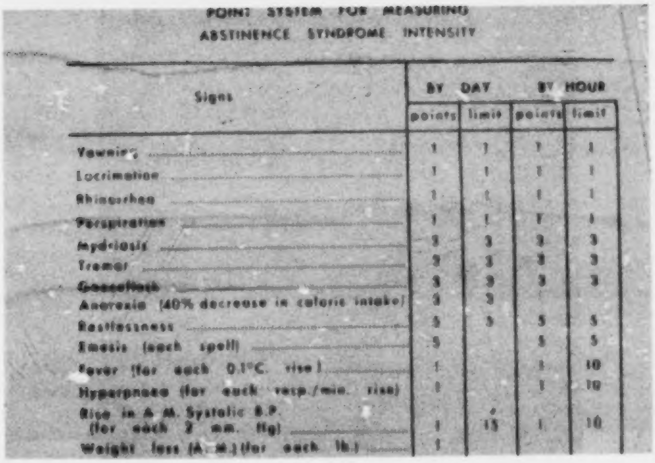
Marked, or +++

Fever
Hyperpnoea
Restlessness
Insomnia

Severe, or ++++

Vomiting
Diarrhea
Weight loss

Figure 1



POINT SYSTEM FOR MEASURING
ABSTINENCE SYNDROME INTENSITY

Signs	BY DAY		BY HOUR	
	points	limit	points	limit
Yawning	1	1	1	1
Lacrimation	1	1	1	1
Rhinorrhea	1	1	1	1
Perspiration	1	1	1	1
Mydriasis	3	3	3	3
Tremor	3	3	3	3
Gooseflesh	3	3	3	3
Anorexia (40% decrease in caloric intake)	3	3		
Restlessness	5	5	5	5
Emesis (each spell)	5		5	5
Fever (for each 0.1°C. rise)	1		1	10
Hyperpnoea (for each resp./min. rise)	1		1	10
Rise in A.M. Systolic B.P. (for each 2 mm. Hg)	1	15	1	10
Weight loss (A.M.) (for each lb.)	1			

Figure 2

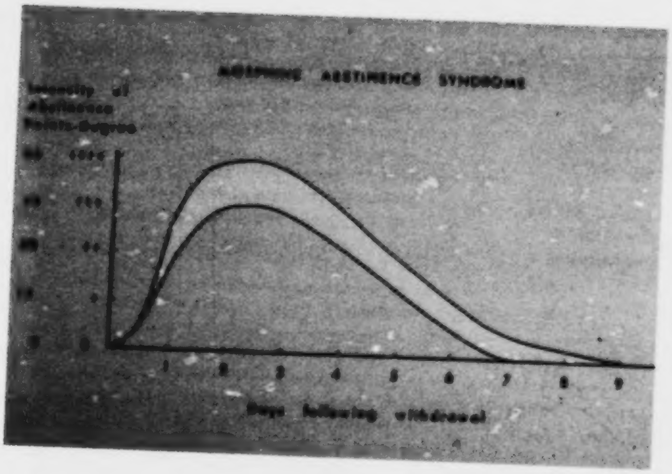


Figure 3

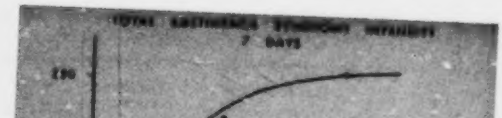


Figure 4

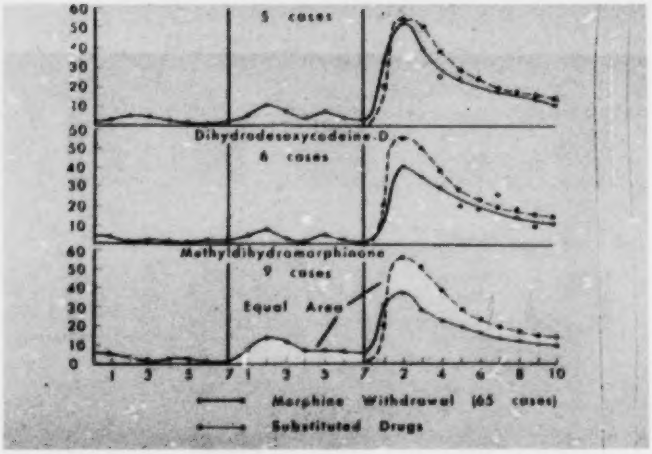


Figure 5

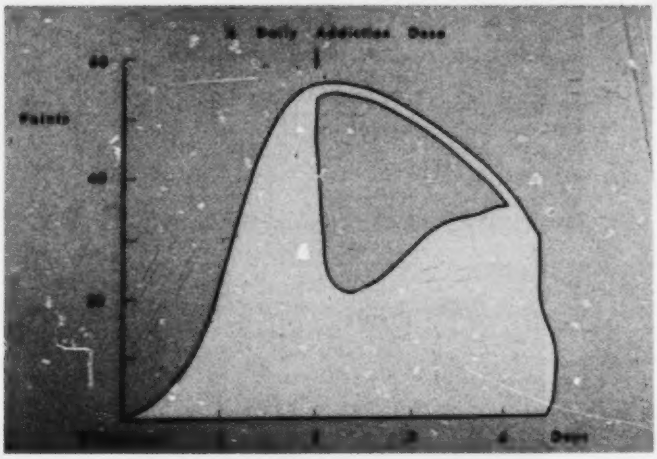


Figure 6

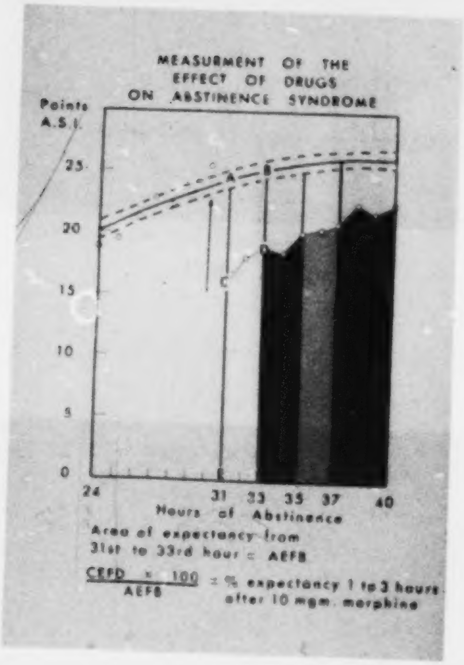

MEASURMENT OF THE EFFECT OF DRUGS ON ABSTINENCE SYNDROME
Points A.S.I.
25
20
15
10
5
0
A
B
C
D
E
F
24
31
33
35
37
40
Hours of Abstinence
Area of expectancy from 31st to 33rd hour = AEFB
CEFD × 100 / AEFB = % expectancy 1 to 3 hours after 10 mgm. morphine

Figure 7

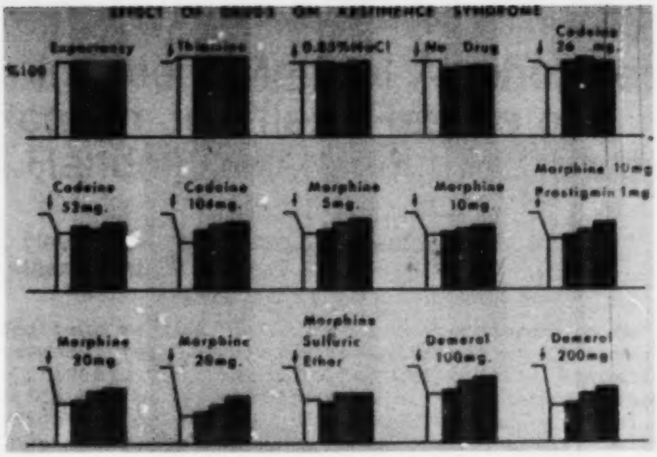


Figure 8

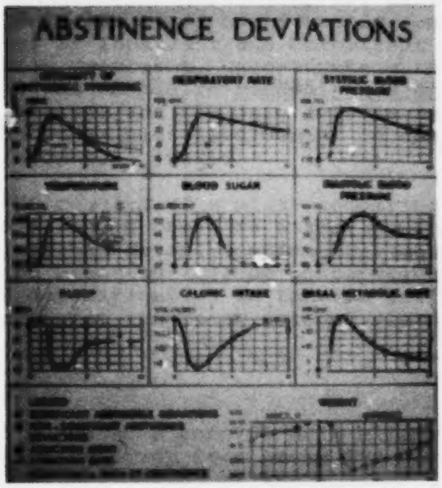


Figure 9

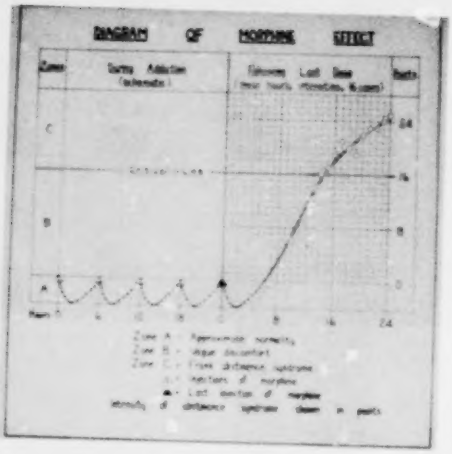


Figure 10

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Is Inpatient Medical Alcohol Detoxification Justified: Results of a Randomized, Controlled Study

M. Hayashida, A. Alterman, T. McLellan, S. Mann,
I. Maany and C. O'Brien

Inpatient medical detoxification continues to be the commonest form of alcohol detoxification (Sausser, et al., 1982). Yet, there is indication that detoxification can be accomplished in most patients without medication or medical personnel (Sparadeo, et al., 1982; Annis, 1979), in a nonmedical, "social-setting" (Sparadeo, et al., 1982; Annis, 1979) or on an ambulatory, outpatient basis (Sausser, et al., 1982). Because of its low cost (Sparadeo, et al., 1982), outpatient alcohol detoxification could be an attractive alternative if it can be shown to be comparable in effectiveness. However, to our knowledge, only one study has directly compared the effectiveness of inpatient and outpatient alcohol detoxification treatment. Unfortunately, the investigator was unable to randomly assign patients to the two treatments and the only outcome measure obtained was subsequent involvement in rehabilitation treatment (McGovern, 1983).

We now report results of a study comparing patients assigned on a random basis to either inpatient or outpatient medical detoxification treatment. The results to be reported include comparative data on the efficacy of detoxification in the two settings as well as one month post-treatment outcome status of patients.

METHODS

Subjects: The subjects were 90 male alcoholic veterans recruited for the study from among 280 veterans requesting detoxification at the Philadelphia VA Medical Center's Alcoholism Treatment Unit from March through September 1985. These veterans exhibited sufficient evidence of alcohol withdrawal (AW), as defined by DSM-III, to qualify for outpatient

detoxification, but did not exhibit severe AW, or serious medical or psychiatric symptomatology requiring immediate hospitalization. All patients were able to comprehend medication instructions and agreed to participate in a randomized assignment study after being informed of its details.
They did not differ significantly in sociodemographic and alcohol-related characteristics from the typical patient seeking detoxification at the Medical Center.

Determining Need for Detoxification: Patients requesting detoxification were first screened by a Central Intake worker who obtained general background information and were then evaluated by a psychiatrist. The physical examination, diagnosis of AW, a breathanalysis, i.e. blood alcohol concentration (BAC), and a modified version of the Selected Severity Assessment (SSA), developed by Gross et al (1973) to assess the severity of AW symptoms, were completed by the psychiatrist.

Randomized Treatment Assignment Procedure: The psychiatrist described the research to eligible patients and obtained informed consent. An independent research technician then randomized the patient to either inpatient or outpatient treatment. Forty-nine subjects were successfully randomized to the outpatient detoxification and 41 were assigned to inpatient treatment.

Following the randomized treatment assignment the technician administered the Michigan Alcoholism Screening Test (MAST), Severity of Alcohol Dependence Questionnaire (SADQ), Beck Depression Inventory (BDI) and the Symptom Checklist-90 (SCL-90), instruments designed to measure severity of alcoholism and alcohol dependence, depression and mood, respectively. The Addiction Severity Index (ASI), which assesses the patient's history and functioning with respect to seven life areas- medical, alcohol, drug, employment, legal, social and psychological- was administered on the following day by a research technician. One month post-discharge status was ascertained using the abbreviated ASI designed for follow-up purposes. This instrument was slightly modified to include more detailed information on alcoholism treatment received during the follow-up period. The follow-up interviews were conducted by trained technicians who were blind to patient status.

Detoxification Objectives:
The major objectives of detoxification treatment were to:

1. effectively resolve the acute medical and

psychiatric problems associated with AW, and prevent the more severe symptoms of AW such as seizures or delirium tremens from developing;;

2. disrupt the patient's pattern of abusive drinking; and

3. help the patient to become engaged in the rehabilitation treatment seen as being necessary to establish and maintain alcohol-free living.

Given these immediate objectives, a one month post-treatment follow-up was determined to be the procedure for evaluating the relative effectiveness of the two treatments.

Detoxification Procedures:
Designated inpatients were admitted to the ward that day or on the following morning, while outpatient detoxification was initiated immediately following intake processing at the Alcoholism Outpatient Unit. While there are several variations in the procedures for medical alcohol detoxification (Naranjo and Sellers, 1986), the major components for patients with mild to moderate degrees of AW symptoms are medication with a benzodiazepine to treat the AW and the use of thiamine and multivitamins to correct nutritional deficits that are frequently present as a result of a long period of poor dietary intake. The emergence of more serious symptoms of AW such as seizures and delirium tremens, for the most part, will be effectively prevented by the benzodiazepine administration alone. Discrete measures to correct water and electrolyte imbalances are seldom necessary for this type of patient population.

a. Outpatient detoxification (n=49)- Ambulatory medical detoxification was provided by one of several psychiatrists. The patient was initially evaluated for medical, psychiatric, and social problems. The psychiatrist then reviewed the fundamental aspects of AW and the detoxification procedures with the patient. The need to take medications as prescribed was particularly emphasized.

The typical patient was started on 30mg of oxazepam to be taken qid. and at bedtime on a p.r.n. basis (with the exception of patients with a history of alcohol withdrawal seizures in whom oxazepam was to be taken qid. unless the patient was oversedated), supplemented by multivitamins and thiamine. He was instructed to return for daily evaluations (except weekends) where the AW symptoms (SSA), and his physical and psychiatric status were briefly re-evaluated. Medication was tapered accordingly each day. Limited counselling for social problems was also provided in some cases. However, the patient's need for long-term rehabilitation treatment following detoxification was consistently emphasized.

Criteria for Completion of Detoxification: Successful completion of detoxification was defined in terms of a marked reduction in AW, as measured by the SSA and a negative breathanalysis for three or four consecutive days, depending upon the SSA score; as well as the reduction of the oxazepam dose to zero. A maximum period of two weeks for completion was imposed. The average duration of detoxification was six calendar days (5.5 visits) for treatment completers. Further details on patient response to ambulatory detoxification are provided in another paper (Alterman, et al., in press).

b. Inpatient detoxification (n=41)- The medical detoxification method was similar to that of the outpatient detoxification. In addition, to initiate rehabilitation, the program also offered group counselling, Alcoholics Anonymous meetings, discharge planning, recreational and social therapy. Disulfiram treatment may also be initiated prior to discharge in some cases.

Mean duration of detoxification was 9.8 days, while the total duration of hospitalization was 15.3 days. The additional five days of hospitalization was generally used for the initiation of rehabilitation, additional medical treatment for diagnosed problems, and for protective care pending the availability of a longer term rehabilitation bed at one of the residential programs. Thus, inpatient detoxification treatment was much more intensive and extensive than the outpatient treatment.

RESULTS

Characteristics of Patients at Intake. The characteristics (demographic, alcohol-related, psychological) of the patients assigned to outpatient and inpatient treatment were compared by means of t-tests where the data was continuous or chi square for categorical data. The outpatierts and inpatients did not differ significantly with respect to the more standard demographic characteristics (age, race, education, marital status, etc.). The two groups also did not differ in the severity of alcoholism (MAST), alcohol dependence (SADQ), or AW (SSA) at intake. The psychological status of the two groups, as reflected by their scores on the Beck Depression Inventory and the Symptom Checklist-90, was not found to differ. The comparative status of outpatients and inpatients at intake was also not found to differ on the seven areas of functioning assessed by the ASI (see Table 1). A group difference was revealed on only one of the individual items shown. That is, the inpatients reported significantly more days intoxicated (24.97 vs. 22.81) in the month prior to treatment than the outpatients ($p < .05$). In general, however, very few group differences were found among the host of variables assessed at intake. The data, therefore, indicate that alcoholics randomly assigned to either outpatient or inpatient detoxification did not differ substantially at intake.

Completion of Detoxification: Sixty-nine percent (34/49) of the outpatients completed detoxification as contrasted with 90% (37/41) of the inpatients. This group difference in treatment completion rate achieved statistical significance (X =5.57, 1 df, p=.018). None of the study patients developed seizures or delirium tremens and none are known to have died during detoxification.

One Month Post-Treatment Outcome. Employing the ASI, one month post-discharge information was obtained for 83.7% (41 of 49) of the outpatients and 92.7% (38 of 41) of the inpatients. The proportion of follow-up evaluations completed did not differ for outpatient and inpatient groups. Analyses of covariance were employed to evaluate group differences at one month follow-up (baseline as covariate), while paired t-tests were employed to examine pre- to post-treatment changes for each group. Group composite scores in seven areas of functioning, along with illustrative items in each of the areas, are described in Table 1. These comparisons indicate that the two groups differed significantly only in one area, medical problems, with outpatients reporting significantly more improvement in medical status than inpatients (e.g., self-report of how many days a medical problem was experienced; how troubled the patient is by medical problems, etc.). Both groups reported a considerable reduction in alcohol related problems and improvement in psychological functioning. For example, with respect to alcohol use, the outpatient group reported a decrease from drinking an average of 24 days a month prior to treatment to almost three days for the one-month post-treatment period, while the inpatients reported a decrease from more than 25 days to less than one day. Similarly, intoxication was reduced from 23 days a month to less than two days for the outpatients and from 25 days a month to less than one day a month for the inpatients. Employment and legal status did not improve; indeed, employment problems worsened to a nonsignificant degree for both groups. Drug use appeared to decline for both groups, but only significantly so for the outpatient group. Thus, patients receiving either inpatient or outpatient detoxification did no appear to differ significantly at the one month post-treatment follow-up.

Alcoholism Treatment During Follow-Up Period. The quantity and form of alcoholism treatment, i.e., inpatient vs. outpatient detoxification or rehabilitation, received by the two groups during the follow-up period were not found to differ.

TABLE 1. ASI SCORES OF OUTPATIENTS AND INPATIENTS AT INTAKE AND AT ONE MONTH POST-TREATMENT FOLLOWUP

	(n=41)			(n=38)			
	OUTPATIENTS			INPATIENTS			
VARIABLES	INTAKE		F-U	INTAKE		F-U	p=[a]
Medic. Composite	0.55	***	0.18	0.53	**	0.37	.04
Days med probl.	14.49	**	4.76	13.06		10.75	.02
Employ. Compos.[b]	0.64	*	0.78	0.70		0.83	ns
Days worked	8.00	*	4.56	4.14		2.75	ns
Dollars earned	400.		306.	264.		96.	.07
Alcoh. Compos.	0.75	***	0.19	0.79	***	0.18	ns
Drinking,days[c]	24.32	***	2.95	25.56	***	0.83	ns
Intox.,days	22.81	***	1.55	24.97	***	0.53	ns
Drug Composite	0.06	*	0.02	0.04		0.01	ns
Days heroin use	0.05		0.03	0.14		0.00	ns
Days cocaine use	0.43		0.13	0.61		0.00	ns
Legal Composite	0.05		0.03	0.08		0.04	ns
Crime,days	0.86		0.40	1.91		0.83	ns
Social Compos.	0.37		0.20	0.38		0.22	ns
Days fam. probl.	2.38		1.38	2.92		1.42	ns
Days soc. probl.	0.78		0.17	0.81		0.86	ns
Psychol.Comp.	0.30	*	0.19	0.37	***	0.20	ns
no. probl. days	12.68	*	6.11	17.29	**	7.75	ns

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a. The intake scores were used as the covariate in the analyses of covariance.

b. Composite scores range from a minimum of 0.00 to a maximum of 1.00.

c. Number of days in past thirty.

* Indicates change from baseline to follow-up (paired t-test) of p<.05; ** indicates change of p<.01; *** indicates change of p<.001.

SUMMARY

The findings of this study revealed relatively few short-term outcome differences for patients meeting study criteria who were randomly assigned to either outpatient or inpatient medical detoxification. Given the higher costs of inpatient treatment, these findings suggest that outpatient detoxification be considered as a meaningful and cost-effective treatment for persons with mild to moderate alcohol withdrawal symptomatology.

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Concurrent Alcohol and Tobacco Use by Women

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INTRODUCTION

There is increasing evidence that drugs from several pharmacological classes (alcohol, opiates and certain stimulants) influence tobacco smoking (see Mello and Mendelson, 1986 for review). An association between smoking and drinking was first observed in studies of alcoholic men (Dreher and Fraser, 1967; Maletzky and Klotter, 1974; Walton, 1972; Mello and Mendelson, 1972) and was clearly demonstrated under clinical laboratory conditions (Griffiths et al., 1976). Smoking behavior was measured over 6 hours when alcohol or a non-alcohol control solution was available. Alcoholic men could consume one drink every 30 minutes and smoke their preferred brand of cigarettes. Access to alcohol consistently induced an increase in cigarette smoking in comparison to placebo control sessions when no alcohol was available. Measures of the number of cigarette puffs and butt weight indicated that this effect was not due to smoking less of each cigarette during drinking (Griffiths et al., 1976).

These findings in alcoholic men have been confirmed (Henningfield et al., 1983, 1984) and extended to male social drinkers (Mello et al., 1980a). When social drinkers were given unrestricted access to alcohol over several days, there was a striking covariation between alcohol consumption and cigarette smoking (Mello et al., 1980a). However, pretreatment with a single dose of alcohol did not consistently influence smoking in social drinkers (Henningfield et al., 1984), but in alcoholics, alcohol pretreatment was associated with a significant increase in cigarette smoking (Henningfield et al., 1983, 1984).

Most studies of drug effects on cigarette smoking have been conducted with men. One goal of the current study was to extend the analysis of the relationship between alcohol consumption and cigarette smoking to women. Cigarette smoking appears to be increasing among young women and it is estimated that about 28 percent of American women smoke daily (see Glynn and Cullen, 1985 for review). Despite evidence of the prevalence and adverse medical consequences of cigarette smoking (Gritz, 1980; A Report of the Surgeon General, 1980; Glynn and Cullen, 1985), there is little objective information available on smoking patterns among women or factors that may influence increases or decreases in tobacco use. This is the first report of daily measures of tobacco smoking by women during 35 consecutive days residence on a clinical research ward. Alcohol was available during 21 of the 35 days and the covariation between drinking and smoking was examined.

METHODS

Subjects: Twenty-four adult female volunteers (26 ± 0.76 years old) with a history of regular tobacco and alcohol use gave informed consent for participation in studies to evaluate the effects of alcohol and tobacco use on reproductive function in an inpatient clinical research study. Volunteer subjects were recruited through advertisements in local newspapers. Subjects were fully informed about the nature and duration of each phase of the study and were told that they could withdraw at any time. Each woman was in good health as determined by clinical and laboratory examinations. Urine screens for drug use and pregnancy tests were performed prior to admission to the research ward and all were negative. Subjects were studied in groups of two to four and lived on a clinical research ward for 35 days.

Smoking and Drinking History: These women had smoked tobacco cigarettes for an average of 10.96 years (±1.02). During the month prior to admission to the study, these women reported smoking an average of 18 cigarettes per day (±1.9). Seventeen women reported that they had tried to stop smoking on at least one occasion. These women had been social drinkers for an average of 6.79 (±0.65) years.

Tobacco and Alcohol Availability Conditions: Subjects were given access to tobacco only for seven days; alcohol and tobacco were concurrently available for 21 days; then only tobacco was available for an additional seven days. Tobacco cigarettes were freely available upon request. Each woman was given her preferred brand of cigarettes. The time of each tobacco cigarette request and the number of tobacco cigarettes smoked each day were recorded. Smoking dynamics (puff frequency, puff volume) were not measured.

Subjects also could choose their preferred type of beverage alcohol. Gin, vodka, scotch, bourbon, beer or wine and mixers were available. Distilled spirits (one 50 ml miniature), beer (one 12 ounce 360 ml can) and table wine (12.5 ounces or 375 ml) each cost the same number of purchase points. Subjects were allowed to buy only one alcohol drink at a time and were not allowed to share drinks. There was no limit on the number of alcohol drinks that subjects could elect to purchase each day.

Alcohol and Money Acquisition Procedures: Operant techniques were used to provide an objective and quantitative measure of performance for two alternative reinforcers, alcohol and money. Subjects could work for money at a simple operant task on an FR

300 (FI 1 sec:S) schedule of reinforcement throughout the study. During the period of alcohol availability, women chose whether to work for alcohol or money each time they activated their operant instrument. Points earned for alcohol and for money were not interchangeable. Subjects had to work at the operant task for about 30 minutes to buy one drink or to earn 50 cents upon completion of the study. Additional details of the operant task, operant manipulanda and computer programming circuitry have been described previously (Mello and Mendelson, 1985; Mello *et al.*, 1987).

Medical Status and Related Measures: Pulse, temperature and blood pressure were measured every eight hours and subjects were weighed every day. Daily medical rounds and periodic physical examinations were complemented by weekly clinical laboratory studies. Pregnancy tests were carried out every week.

RESULTS AND DISCUSSION

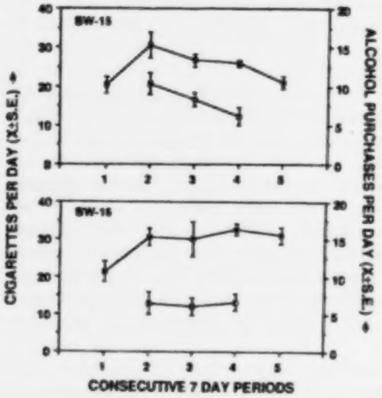
Smoking Patterns: The 24 women were classified as heavy, moderate or occasional tobacco smokers on the basis of the actual number of cigarettes smoked while on the clinical research ward. Seven women who smoked 25 or more cigarettes per day ($\bar{X} = 32 \pm 1.6$) were classified as *heavy* smokers. Eleven women who smoked 15 to 25 cigarettes per day ($\bar{X} = 21 \pm 0.7$) were classified as *moderate* smokers. Six women who smoked 3 to 11 cigarettes per day ($\bar{X} = 8.4 \pm 1.2$) were classified as *occasional* smokers. The average number of cigarettes smoked by each group was significantly different by ANOVA ($P < .001$).

Observed smoking patterns were similar to self-reports of tobacco use before the study. Women classified as heavy smokers reported smoking 25 (± 2.9) cigarettes per day during the month preceding the study. These women had smoked for an average of 13.7 (± 1.06) years. Women classified as moderate smokers reported smoking an average of 20 (± 1.1) cigarettes per day during the month before the study. These women had smoked for 9.9 (± 1.76) years. Women classified as occasional smokers reported smoking an average of 6 (± 1.9) cigarettes per day during the month before the study and had smoked for 9.7 (± 1.9) years.

Concurrent Use of Tobacco and Alcohol: Women who were heavy smokers smoked significantly more during alcohol availability when smoking was analyzed independent of alcohol consumption ($P < .05$). Smoking increased by 5.5 ± 0.95 cigarettes per day over the pre-alcohol baseline. Moreover, there was a striking concordance between variations in alcohol intake and in cigarette smoking. Correlational analysis revealed that smoking and drinking were significantly related ($r = .602$; $P < .01$). The heavy smokers also smoked significantly more after alcohol availability than before drinking ($P < .01$) (34 ± 2.7 cigarettes per day). The heavy smokers also smoked significantly more than the moderate and occasional smokers during all phases of the study ($P < .001$).

Moderate smokers smoked 2.8 (± 0.64) more cigarettes each day during drinking than during baseline but this difference was not significant. Occasional smokers also smoked 0.9 (± 1.62) more cigarettes during drinking than during baseline, but this difference was not significant. Both moderate and occasional smokers acquired an equivalent number of cigarettes before and after alcohol availability. Variations in smoking and drinking were significantly correlated in the occasional smokers (r = .671; P < .001) but not in the moderate smokers.

Analysis of smoking as a function of alcohol intake also showed that women who were heavy and moderate drinkers smoked significantly more during alcohol availability than during the pre-alcohol baseline. Moreover, the alcohol-associated increases in smoking by moderate and occasional drinkers persisted during the post-alcohol period. During the pre-alcohol baseline period, five heavy alcohol users smoked an average of 19 (± 3.4) tobacco cigarettes each day. During alcohol availability, the heavy drinkers drank an average of 7.8 (± .69) drinks per day and increased smoking significantly by 6 (± 1.24) cigarettes per day (P < .01).



Illustrative data for two heavy drinkers who smoked an average of one pack of cigarettes per day during the pre-alcohol baseline are shown in Figure 1. These individual smoking and drinking data show an alcohol dose dependent influence on tobacco smoking. Subject 15 increased average tobacco smoking from 20 to 30 cigarettes per day when she drank an average of 10 (± 1.38) drinks per day. As alcohol consumption declined to 8 (± .92) and 6 (± 1.18) drinks per day, tobacco smoking also decreased from 30 to 26, then 25 cigarettes per day. Subject 16 also increased tobacco smoking from 21 to 30 cigarettes per day when she began to drink over 6 drinks per day. Subject 16's alcohol intake remained at 6 drinks per day throughout the 21 day period and cigarette smoking also remained stable at 31 (± 2.20) cigarettes per day.

Ten moderate alcohol users smoked an average of 21 (± 2.5) tobacco cigarettes per day during baseline. The moderate alcohol users drank 3.85 (± .19) drinks per day and increased smoking significantly during alcohol availability ($P < .05$). The moderate alcohol users also smoked significantly more during the post-alcohol period than during the pre-alcohol baseline ($P < .05$).

Nine occasional alcohol users smoked an average of 16 (± 2.6) tobacco cigarettes per day during the pre-alcohol baseline. These women drank 1.22 (± .21) drinks per day and increased smoking to 17 (± 2.9) cigarettes per day. As the study progressed, the occasional alcohol users continued to smoke more cigarettes.

Distribution of Intervals Between Successive Cigarettes: The alcohol-related increases in the number of cigarettes smoked each day were also associated with significant changes in the overall rate of cigarette smoking. Women requested more cigarettes at intervals of 11 to 20 min during alcohol availability than during the pre-alcohol condition when cigarettes were usually smoked at intervals of 21 to 30 or 31 to 40 min. These pre-alcohol inter-cigarette intervals are similar to previous observations in three male heavy smokers when puff duration and inter-puff intervals were controlled (26.11 ± 1.58 to 37.28 ± 2.43 min) (Griffiths *et al*., 1982).

The shift in the distribution of inter-cigarette intervals during drinking is comparable to that previously observed in men maintained on buprenorphine (Mello *et al*., 1985) and on heroin (Mello *et al*., 1980b, 1985). This smoking pattern is consistent with the timecourse of nicotine metabolism, since plasma nicotine levels peak within 10 min and fall precipitously in about 20-30 min (Armitage, 1978; Gritz, 1980; Jaffe and Jarvik, 1978). These findings with alcohol extend our previous observations that increased cigarette smoking during opioid drug use occurs primarily at shorter inter-cigarette intervals rather than being distributed equally across the range of intervals studied (Mello *et al*., 1980b, 1985).

These data in women confirm and extend previous reports of an association between cigarette smoking and drinking by alcoholic men (Griffiths *et al*., 1976; Henningfield *et al*., 1983; Mello and Mendelson, 1972) and male social drinkers (Mello *et al*., 1980a). Although all women smoked more during alcohol availability, the magnitude of the increase was related to the relative smoking and drinking level. Heavy smoking and heavy and moderate drinking were associated with greater increases in smoking during drinking than moderate and occasional smoking and/or occasional alcohol use.

The mechanisms by which alcohol affects cigarette smoking are unclear. Drugs from several pharmacologic classes including opioid agonists and mixed agonist-antagonists and stimulants have also been shown to be associated with increased cigarette smoking (Mello *et al*., 1980b, 1985; Schuster *et al*., 1979; Mello and

Mendelson, 1986). Consequently, it is difficult to postulate that any specific pharmacological effect accounts for increased cigarette smoking during intoxication.

There remain many complex and as yet unresolved questions concerning the nature of the reinforcer in polydrug use and abuse. We have suggested elsewhere that a stimulus self-administration framework may be a useful way to think about polydrug use of substances with conflicting or antithetical behavioral effects (Mello, 1977, 1983). The reinforcer for drug use may be a change in state, and the direction of that change may be less important than the occurrence of the change itself (for review, see Mello, 1983; Mello and Mendelson, 1986). The reinforcing properties of cigarette smoking and how these are influenced by simultaneous use of other substances remains to be clarified.

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The Syndrome Concept of Alcohol and Drug Dependence: Results of the Secondary Analysis Project

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INTRODUCTION

This report describes the accomplishments of a multi-center collaborative project formulated within the context of the ongoing WHO/ADAMHA program on Classification and Diagnosis. The goal of this project has been to provide empirically-based research findings relevant to the improvement of diagnostic systems currently used for the classification of alcohol and drug dependence syndromes. Of particular interest to this investigation was the detailed examination of the drug dependence syndrome (DDS) concept (Edwards et al., 1981), its patterning in clinic samples of opiate and alcohol dependent persons, its generalizability to diverse cultural settings, its relation to certain antecedent and concurrent variables, and its value as a predictor of treatment response. The goals of the project have been accomplished by re-analysis of nine existing data sets obtained from recent treatment evaluation studies conducted in the U.S., England, Canada and France. In view of the potential commonalities existing within these data sets, collaborating investigators coordinated a series of secondary analyses designed to provide a preliminary evaluation of the WHO dependence model. The analyses examined: 1) commonalities in the internal consistency and factor structure of dependence elements across substances (opiates and alcohol) and across cultural groups; 2) the relative independence of the hypothesized dependence syndrome from other kinds of physical, social and psychological disability; 3) the relation between severity of dependence and rapidity of relapse.

METHODS

Detailed description of the study samples and research methodologies is provided in the published reports of the collaborating investigations (Babor, Cooney and Lauerman 1987; Bray et al., 1981; Kosten et al., in press; Woody et al., 1983; Skinner 1986; Kosten et al., 1986). These studies evaluated alcohol and/or drug dependent patients at admission to treatment and, in many cases, again at follow-up. Conducted by senior investigators in Great Britain, France, the United States, and Canada, these studies have produced data sets representing more than 16,000 patients. The secondary analyses of these data sets fall into three categories: a) internal consistency reliability analyses of dependence syndrome items; b) factor analyses of dependence syndrome measures; and c) analysis of the predictive validity of syndrome elements.

RESULTS

Internal consistency analysis. A major assumption of the dependence syndrome concept is that a common core of behaviors, cognitions and physiological symptoms tends to co-vary in frequency and intensity. The common syndrome elements include narrowing of the substance use behavioral repertoire, increased salience of substance-taking behavior, increased tolerance, repeated withdrawal symptoms, subjective compulsion to use the substance, use to relieve withdrawal, and increased liability to readdiction. In order to test this hypothesis, internal consistency reliability analyses were conducted on data from 15 samples of opiate users and alcoholics. The results of those analyses are summarized in Table 1. Items consistent with the WHO dependence model were selected from the scales indicated on the table. Where data were available on females, these have been considered separately to provide additional replications. The samples are predominantly alcoholic patients and all items analyzed pertain to the hypothetical elements of the alcohol or drug dependence syndrome. The results indicate a high degree of internal consistency reliability across relatively disparate samples of alcoholics, opiate users and polydrug users as indicated by the high Cronbach's alphas.

The above analyses of internal consistency strongly suggest that the dependence syndrome items form cumulative, unidimensional scales. As a further test of unidimensionality, Kosten et al., (in press) attempted to form Guttman scales for each type of drug from 10 items derived from the Structured Clinical Interview for DSM-III-R (SCID). Most of the drugs demonstrated good approximations of "perfectly" unidimensional and cumulative scales. The coefficients were 0.85 or above for all drugs. This indicates that within each type of drug, higher scores are consistent with more severe dependence. The items associated with a more severe syndrome differed across the various drugs. For example, opiate users reporting "withdrawal symptoms" almost always reported every other item of the dependence syndrome, but users of other substances often reported this item with few other dependence symptoms. At the other extreme, opiate users often reported "using more opiates than they intended" along with few or no other dependence items. Other types of substance users usually reported this item in conjunction with most other items in the syndrome.

Factor analyses. A second test of the syndrome hypothesis was to apply factor analysis to those data sets having an adequate representation of items measuring both dependence elements and problems related to alcohol or drug use. Factor analyses have been conducted on three samples (Farmington, Tours, Toronto),

Table 1. Results of internal consistency reliability analyses.

Sample	Instrument	Sex, Sample Size & Primary Diagnosis of Respondents	Number of Items	Type of Dependence	Cronbach's Alpha
UConn Farmington	LSXMO[a]	197 male alcoholics	14	alcohol	.86
		64 female alcoholics	14	alcohol	.81
	MAST[b]	156 male alcoholics	5	alcohol	.70
		58 female alcoholics	5	alcohol	.62
Saint Andrew's England	Hilton[c]	53 male alcoholics	17	alcohol	.88
		44 female alcoholics	17	alcohol	.86
Tours France	LSXMO[a]	177 male alcoholics	18	alcohol	.89
		36 female alcoholics	18	alcohol	.90
Yale New Haven	MAST[b]	413 male opiate users	5	alcohol	.82
		130 female opiate users	5	alcohol	.63
Yale New Haven	SADS[d]	413 male opiate users	7	alcohol	.78
		130 female opiate users	7	alcohol	.76
	SCID[e]	41 mental patients	10	alcohol	.91
		42 polydrug users	10	sedatives	.85
		54% males, 46% females	10	hallucinogens	.90
			10	stimulants	.91
			10	cannabis	.83
			10	cocaine	.95
			10	opiates	.98

[a] Last Six Months Drinking Questionnaire
[b] Michigan Alcoholism Screening Test
[c] Hilton Drinking Questionnaire
[d] Schedule for Affective Disorders and Schizophrenia
[e] Structured Clinical Interview for DSM-III-R

providing good support for the statistical independence of dependence symptoms and problem indicators. The results of those analyses are described in papers by Babor, Lauerman and Cooney (1987), Skinner and Goldberg (1986), and Kosten *et al.*, (in press).

Predictive validity. Additional analyses tested the relative predictive power of pre-treatment dependence in relation to a variety of other intake variables in accounting for the severity of dependence at post-discharge follow-up among those patients who attempted to drink or use opiates.

One analysis tested the hypothesis that the greater the degree of alcohol dependence prior to admission to treatment, the more likely the syndrome is to be reinstated once drinking is initiated following a period of abstinence. The results, summarized in Babor, Lauerman and Cooney (1987) showed that the strongest and most consistent predictors of severity of dependence at 12-month follow-up among males ($N=150$) are measures reflecting recent and lifetime dependence history. Although pre-treatment dependence was found to be a significant predictor for females ($N=47$), the degree of social problems at intake was a stronger correlate, and the lifetime dependence symptom count from the DIS did not predict reinstatement in females.

Another hypothesis derived from DDS theory concerns the role of craving as a mediator of relapse. It has been suggested that craving is a cognitive label applied to conditioned responses precipitated by alcohol-related stimuli. If craving is a consequence of an alcoholic's dependence history, then reports of the frequency and intensity of craving should be greatest in persons scoring higher on our dependence measures. In order to test this hypothesis, we correlated the intake dependence measures with two measures of craving at one-year follow-up. In order to control for the confounding influence of concurrent drinking on craving, we analyzed data only from patients who remained abstinent from alcohol during the entire follow-up period. The results indicate that the severity of recent pre-treatment dependence predicts the intensity of post-treatment craving in both males ($r=.33$, $p < .05$) and females ($r=.55$, $p < .05$).

A final series of analyses was designed to test the reinstatement hypothesis in samples of opiate users studied by researchers at the University of Pennsylvania (UPenn, Woody et al., 1983), Yale University (Kosten et al., 1986), and Research Triangle Institute (RTI, Bray et al., 1981). The primary dependent measures of interest to these analyses were the Drug and Alcohol Composite scores from the Addiction Severity Index (ASI; McLellan et al., 1981). These composites are computed from questions about recent frequency of use, money spent on drugs or alcohol, and number of days in last month bothered by drug or alcohol problems, such as craving, withdrawal, or impaired control. ASI composites representing alcohol and drug severity at intake and six-month follow-up evaluation were correlated within three study samples: UPenn males ($N=94$), Yale males ($N=71$), Yale females ($N=27$). The results indicated that drug severity at intake predicts drug severity at follow-up only in the UPenn sample ($r=.34$, $p < .01$). The relationship between intake and follow-up alcohol severity was replicated in both male samples, however. Re-analysis of the RTI data set, using two measures of reinstatement (rapidity of return to daily use and any return to daily use) in a logistic regression analysis, was based on patients not participating in methadone maintenance programs who relapsed to opiate use during the one-year follow-up period ($N=208$). The results revealed few significant predictors of either measure of reinstatement and no evidence that indicators of prior dependence, such as treatment history or number of drugs used, predicted reinstatement.

DISCUSSION

A major assumption of the DDS concept is that the syndrome elements tend to co-occur in frequency, intensity and duration. Another assumption is that syndrome elements are statistically as well as conceptually independent of disabilities consequent to alcohol or drug use. The results of the internal consistency reliability analysis conducted on various item sets provided strong support for the syndrome hypothesis. Cronbach's alphas were consistently high across samples regardless of substance (opiates, alcohol), gender (males, females) or cultural identification (English, American, French). Kosten et al.'s,(in press) Guttman Scale analysis of identical items rated across six substances provided additional support for the unidimensionality of the syndrome elements.

Factor analysis of the alcohol dependence items in samples of American and French alcoholics demonstrated statistical independence from scales measuring alcohol-related problems. Skinner's and Goldberg's (1986) factor analysis of the DAST (Drug Abuse Screening Test) items provided a preliminary replication of these findings in a sample of drug users. A three-item factor, labeled dependence, was defined by items measuring impaired control over drug intake, compulsion to use the substance, and drug withdrawal symptoms. The findings are consistent with those obtained from alcoholics, opiate addicts, and polydrug users studied by Kosten et al.,(in press) and McLellan et al.,(1981).

The analyses described in this paper also attempted to evaluate the usefulness of DDS measures as predictors of dependence severity following posttreatment use of either alcohol or drugs. The results provide some support for the predictive validity of alcohol dependence measures, not only in persons whose primary diagnosis is alcoholism (UConn sample), but also among opiate addicts (Yale and UPenn samples). While the relatively high prevalence of alcohol abuse in the opiate addict samples makes these groups a logical source to test the generalizability of the findings obtained from the alcoholics, it should be noted that the nature of the follow-up methodology employed does not permit a clear statement about the extent to which alcohol dependence was actually reinstated. These correlations may merely reflect the continuation of heavy drinking by the opiate addicts during the posttreatment period without any significant period of alcohol abstinence during treatment.

Although the ASI drug severity score was a significant predictor of posttreatment dependence severity in the UPenn sample, the correlations found in the other samples were not significant. These mixed results could be influenced by a variety of extraneous factors that make the DDS reinstatement hypothesis difficult to test in samples of opiate addicts. The first difficulty is the measurement of dependence. In the present analysis, we relied primarily on the drug severity composite from the ASI, a measure that only approximates the content of the drug dependence construct. Second, it was difficult to control for the opportunity to reinstate dependence, since this depends on such factors as initiating opiate use following a period of abstinence. Many drug-treatment subjects in these analyses never truly abstained from opiates since they were actually enrolled in methadone maintenance programs. Under these circumstances, it is surprising that a significant relationship was observed in the UPenn sample.

In conclusion, the DDS concept provides the basis for a relatively unambiguous set of hypotheses about the conditions under which alcoholics and opiate addicts re-acquire dependence after a period of abstinence. Instead of conceiving relapse as a symptom of some underlying personality problem, it focuses attention on the patient's maladaptive habit patterns and the environmental as well as interoceptive stimuli that precipitate reinstatement of dependence. The present research suggests that the DDS concept may have merit as a theory of drug and alcohol dependence, but should be subjected to a more rigorous program of research aimed at better operational measures and more intensive hypothesis testing, especially in samples of drug users.

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Placebo Challenge: Effects Following Repeated Alcohol vs. Placebo Administration

M. McCaul, J. Turkkan and M. Stitzer

When the physiological and subjective effects of a drug such as ethanol are repeatedly paired with stimuli in the natural environment, drug-related conditioned responses may come to be elicited by these previously neutral stimuli. Such conditioned responses are thought to play an important role in triggering craving and precipitating relapse to drug use following periods of abstinence. While much of the objective evidence of conditioning during repeated drug administrations has been obtained in animal studies, there have been a number of demonstrations of the operation of conditioning in humans.

Some research with alcoholics has reported differences in heart rate, skin conductance and subjective response measures when subjects were given controlled exposure to the taste of alcoholic versus nonalcoholic beverages in doses that were insufficient to produce direct drug effects (Baker and Cannon, 1979; Cannon and Baker, 1981; Kaplan et al, 1985); however, other studies have failed to replicate these findings. In a recent study with social drinkers, Newlin (1985) obtained significant increases in heart rate and decreases in skin conductance depending on subjects' identification of near-beer as an alcoholic or nonalcoholic beverage. He suggested that the sight, taste and smell of beer alone were not sufficient as conditioned stimuli but rather the "experiential feeling of intoxication" was necessary for demonstration of conditioned responses.

The present study examined the effects of a placebo drink in alcoholic subjects following repeated administration of alcohol or placebo drinks in a laboratory environment. One group of subjects received active alcohol during sessions 1 through 4 and placebo on day 5; the second group received placebo throughout sessions 1 through 5. We hypothesized that subjects receiving placebo in an environment previously associated with alcohol availability would exhibit conditioned responses as compared with subjects in the placebo control group. Further, we predicted that these conditioned responses would be opposite in direction to responses obtained during the active drink sessions.

METHODS

Subjects. Subjects in both groups were alcoholics who met the following selection criteria: five or more years heavy alcohol use, 10 or more drinks on 3 - 7 days per week, one or more dependence symptoms such as tremors, morning drinking or blackouts, and either prior treatment for alcohol abuse or alcohol-related social, legal or employment problems. Twelve subjects were assigned to the active alcohol condition and twelve subjects were assigned to the placebo condition. There were no differences in demographic characteristics or in the number and type of alcohol-related problems between the two groups.

Since subjects were current heavy drinkers at the time of study enrollment, subjects were hospitalized for a minimum of three days before their first experimental session and remained hospitalized throughout the research protocol. All subjects received thorough medical and psychiatric screens and provided signed informed consent prior to experimental assignment. Subjects were paid for their participation.

Procedures. Subjects participated in five successive daily sessions each of which consisted of two methodologically distinct data collection periods. At the beginning of each session, subjects received a variable number of randomized-block presentations of three types of stimulus trials. Stimulus trials included taste, sight and smell of alcohol, pepper juice or water. These data are not included in this paper.

At the conclusion of the daily stimulus trial series, subjects in the alcohol condition received 1.7 g/kg ethanol on days 1 - 4 and placebo on day 5. Subjects in the placebo condition received placebo drinks across days 1 - 5. Orange juice was mixed with the appropriate dose of alcohol to maintain a constant volume of 16 oz; all drinks were delivered in a thermos surrounded by alcohol-soaked gauze. A mouth-piece filled with 0.2 ml alcohol was attached to the straw in order to deliver an initial strong taste of alcohol in both drink conditions (Mendelson et al, 1984). Subjects were instructed to consume the drink during a 5-minute period. The research assistant and subjects were blind to experimental assignment.

Physiological measures were collected continuously throughout the drink period and the subsequent 20-minute interval. Heart rate was recorded as interbeat interval using chest EKG electrodes; the signal was amplified using a Beckman polygraph. Skin temperature was measured using a skin surface thermistor placed on the middle finger of the nondominant hand. Skin conductance was recorded using an Autogen 3000 from two finger-tip electrodes placed on the nondominant hand. In addition, five self-report analog questions were completed a total of four times at 5-minute intervals post-ingestion. Analog items were displayed on a computer screen and responded to using a joystick which advanced a cursor on the screen. Analog items included: high, good effects, bad effects, like drink and craving.

Physiological measures were analyzed using repeated measures analyses of covariance with baseline (1-minute pre-drink) as the covariate. Self-report analog items were analyzed with repeated measures analyses of variance. For both physiological and subjective data, factors included group (alcohol vs. placebo), days (separate analyses were conducted using data from all days and using only day 1 vs. day 5 data) and minutes. Planned comparisons were conducted when significant effects of days or minutes were observed.

RESULTS

Physiological Measures. As seen in Figure 1, heart rate remained relatively stable throughout the 20-minute post-ingestion period for both the alcohol and placebo groups on day 1. In contrast on day 5, heart rate decreased following ingestion of the placebo drink in subjects who had consistently received alcohol on days 1 - 4. For the placebo group, there was no change in heart rate response during the post-ingestion period from day 1 to day 5. Comparing heart rate on day 1 versus day 5, there was a significant days X group interaction ($p<.05$). Planned comparisons indicated that on day 1, heart rate in the alcohol group was significantly less than in the placebo group only during minute eleven through fifteen post-ingestion ($p<.05$). Whereas on day 5 follow-

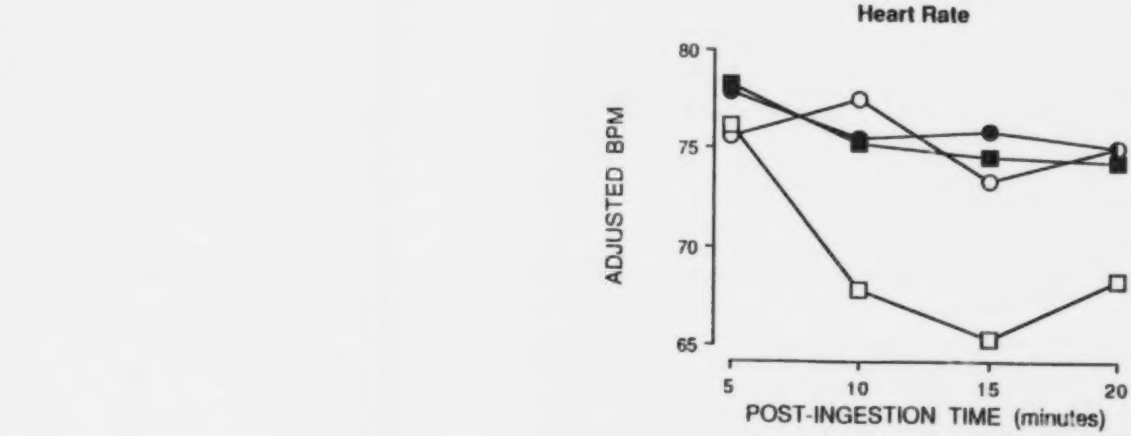

FIGURE 1. Mean heart rate following ingestion of alcohol or placebo over successive minutes post-ingestion. Subjects in the alcohol group received 1.7 g/kg ethanol on day 1 (○) and placebo on day 5 (□). Subjects in the placebo group received placebo drinks on day 1 (●) and day 5 (■). Data were averaged over a 5-minute interval for 12 subjects per group.

ing placebo administration, heart rate was significantly less in the alcohol group than in the placebo group from minute 6 post-ingestion to the end of the 20-minute measurement period (p<.01).

As shown in figure 2, skin conductance on day 1 was significantly higher in the group receiving alcohol than in the placebo group from minute 6 post-ingestion to the end of the 20-minute measurement period (p<.01). In contrast, on day 5 following placebo administration in both groups, skin conductance was significantly lower in the group previously receiving alcohol than in the group receiving placebo throughout all five sessions. This difference in skin conductance was significant from minute 11 post-ingestion to the end of the measurement period (p<.05). For skin conductance there was a significant group X days X minute interaction (p<.0001).

There were no significant day 1 vs. day 5 effect on skin temperature; however, across all days, there was a significant group X days X minute interaction (p<.02). As shown in Figure 3, skin temperature on day 1 showed little systematic change from baseline following ingestion of alcohol or placebo over the 20-minute measurement period. On days 2 - 4 following active alcohol, skin temperature increased above day 1 alcohol levels and also above placebo levels during the sessions. On day 5, skin temperature

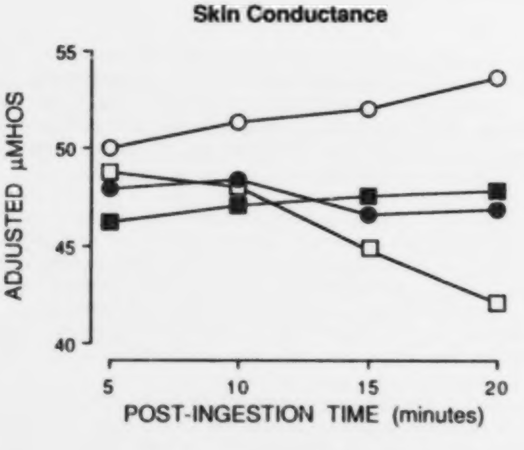

FIGURE 2. Mean skin conductance following ingestion of alcohol or placebo over successive minutes post-ingestion. Subjects in the alcohol group received 1.7 g/kg ethanol on day 1 (○) and placebo on day 5 (□). Subjects in the placebo group received placebo drinks on day 1 (●) and day 5 (■). Data were averaged over a 5-minute interval for 12 subjects per group.

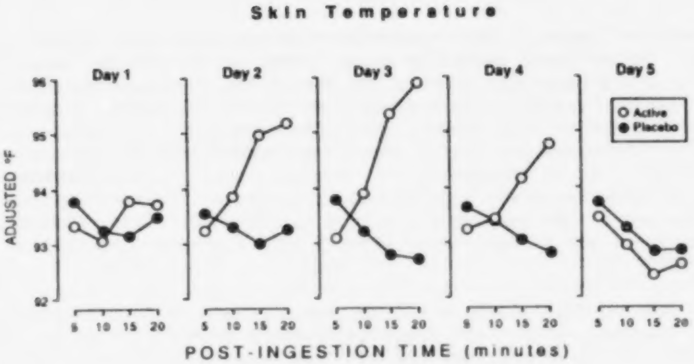

FIGURE 3. Mean skin temperature following ingestion of alcohol or placebo over successive minutes post-ingestion. Subjects in the alcohol group received 1.7 g/kg ethanol on days 1 - 4 and placebo on day 5. Subjects in the placebo group received placebo drinks over days 1 - 5. Data were averaged over a 5-minute interval for 12 subjects per group.

showed a similar decrease across the 20-minute measurement period for both groups. Post-hoc comparisons of skin temperature across days revealed a significant difference between day 2 and day 5 ($p<.05$) and between day 3 and day 5 ($p<.01$) for the alcohol group only; there were no significant differences in skin temperature across days for placebo subjects.

Self-report Measures. Similar results were obtained for three out of five self-report measures. Scores for high and good effects were higher in the alcohol group than in placebo group across sessions 1 - 4. In contrast, on day 5 following placebo administration, scores were similar for the two groups. Comparing day 1 versus day 5, there were significant group X days X minute interactions for high ($p<.03$) and good effects ($p<.007$).

Finally, there was a significant days effect ($p<.04$) and a marginal group X days interaction ($p<.08$) for craving. As shown in figure 4, craving was lower in the alcohol group than in the placebo group on day 1. Craving increased from day 1 to day 4 in the alcohol group even though subjects were receiving a constant, high dose of alcohol daily. On day 5 following placebo administration, craving scores for the alcohol group were elevated above the placebo group. There were no systematic changes in craving in the placebo group over days.

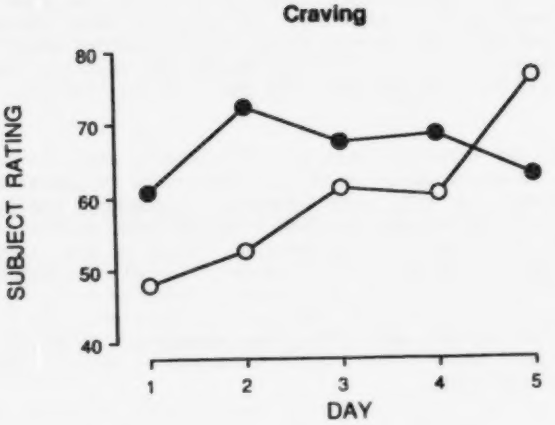

FIGURE 4. Mean subject ratings of alcohol craving over days. Subjects in the alcohol group received 1.7 g/kg ethanol on days 1 - 4 and placebo on day 5. Subjects in the placebo group received placebo drinks over days 1 - 5. Each point is the average of 4 ratings made at 5-minute intervals post-ingestion, averaged over 12 subjects per group.

DISCUSSION

A placebo challenge resulted in different physiological and subjective responses depending on whether alcoholic subjects had received earlier repeated administrations of alcohol or placebo in the laboratory. Specifically, following a placebo challenge, heart rate and skin conductance were significantly lower in subjects previously receiving alcohol than in subjects previously receiving placebo. This effect on skin conductance was opposite in direction to the significant increase in skin conductance following active alcohol administration on day 1. In addition, craving scores following a placebo challenge were higher for subjects previously receiving alcohol than for subjects previously receiving placebo.

Results on all three measures for the alcohol group only are consistent with Siegel's model of conditioned compensatory responses to repeated drug administration (Siegel, 1980). According to this model, repeated drug administration results in the development of drug-opposite conditioned responses which then serve to offset the direct actions of the drug. While the results of many animal studies on drug conditioning and tolerance have supported this model, the results from human research have been less consistent. As mentioned earlier, a recent study by Newlin (1985) has suggested that exposure to alcohol-related stimuli alone may not be sufficient to elicit conditioned responses in humans, rather subjects must experience alcohol-like effects in order to demonstrate compensatory responses. The present data further suggest that subjects may exhibit conditioned responses when the environment signals alcohol availability but not when the same environment has been associated with the non-availability of alcohol.

These findings are important both methodologically and clinically. In terms of experimental methods, conditioned responses may be smaller and more variable using laboratory procedures which do not explicitly include alcohol availability; to date, most of the human research on alcohol conditioning has relied on the taste, smell and/or sight of alcohol. In terms of clinical application, the data support the importance of relapse prevention training in treating alcoholic clients since they may not experience the full impact of physiological and subjective responses to alcohol-related cues until their return to environments in which alcohol continues to be available.

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May be obtained from the senior author on request.

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Interactions Between Social Behavior and Smoked Marijuana

R. Foltin, M. Fischman and J. Brady

A previous report from this laboratory (Foltin et al., 1986) described the effects of smoked marijuana on contingency-controlled behavior under conditions that involved the maintenance of a low probability private work performance by access to a high probability work task. The results of that study, as with earlier reports describing marijuana effects on behavior maintained by monetary contingencies (Mendelson et al., 1976; Miles et al., 1974), showed no significant change in the required instrumental performances following drug administration. Interactions between smoked marijuana and contingency-controlled behavior were suggested however, by the finding of a somewhat surprising decrease in the contingent high probability work performance under active marijuana conditions described by Foltin et al., (1986). The present report describes a study that extended the analysis of interactions between marijuana and performance to behavioral contingencies involving social and recreational activities. Specifically, the effects of smoked active and placebo marijuana cigarettes were compared in the presence and absence of contingencies that required performance of low probability individual behaviors to access high probability social interactions.

METHOD

Subjects. Six healthy adult male volunteers ranging in age from 21 to 35 participated in two long-term residential experiments. All subjects were current marijuana users. Subjects received extensive medical and psychiatric examinations prior to research participation and signed consent forms that provided a detailed explanation of the experimental procedure.

Laboratory. The studies were conducted in a self-contained human residental laboratory environment that has been previously described in detail (Brady et al., 1975). The three identical private rooms (2.6 x 3.4 x 2.4 m) are similar to facilities, bed, desk, chair, and other furnishings. The social living area (4.3 x 6.7 x 2.7 m) is equipped with tables, chairs, sofa beds, and a complete kitchen facility. The workshop (2.6 x 4.1 x 2.7 m) contains an exercise area and a washer-dryer combination. A common bath serves the social living area and the workshop. Access to the exterior walls of the laboratory is provided by a corridor between the residential chambers and the external building shell that permits transfer of supplies and materials through two-way storage facilities accessible from both sides.

One subject resided in each of the three private efficiency apartments and all had access to group areas at programmed times. All subjects remained within the residential laboratory environment throughout the duration of the study. A networked computer system with terminals in each room provided all contact between subjects and experimenters. Subjects were monitored from an adjacent control room using a video and audio monitoring system and a computerized behavioral observation program (Bernstein & Livingston, 1982).

Standard Day. The day consisted of three sections: a private work period, a performance test, and a period of social access. Subjects were awakened at 09:00, ate breakfast and had a work period from 10:00 to 15:00. During the work period, subjects were required to remain in their private rooms and engage in one of four performance tasks. Subjects were tested in a performance battery from 15:30 to 16:30. During the remainder of the day subjects either remained in their private rooms or used the facilities in the social area. The day ended at 24.00.

Procedure. The design for the experiment is presented in Table 1. The protocol involved both baseline and contingency periods, with placebo and active marijuana cigarette smoking superimposed on each of these conditions. During baseline conditions ("BASE" - Table 1), subjects engaged in activities in the absence of restrictions. Each behavioral activity was monitored continuously via the computerized observational system and time spent in each activity was recorded for each subject. The resultant time-based behavioral hierarchies determined the contingency conditions ("CONTGY" in Table 1)

Table 1

EXPERIMENTAL DESIGN

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Drug	NS					MJ			PBO				MJ			PBO		
Social			BASE			CONTGY			BASE			CONTGY			BASE			BASE

during which subjects had to spend time doing the least preferred activity. A contingency relationship was determined for each subject using the response-probability procedure of Premack (1965). This required that subjects engage in four times the amount of their least preferred baseline activity (the instrumental activity) in order to maintain baseline levels of their most preferred activity (the contingent activity). During contingency periods, lack of availability of the contingent activity was indicated by illumination of a red light in each subject's room and the social area. Time earned for the contingent activity accumulated as time was spent performing the instrumental activity. As long as there was time accumulated for the contingent activity, each subject could use it as he chose. Time earned was carried over each day for the entire contingency period. Subjects were not told the duration of the contingency, and each afternoon they were given written instructions describing the contingency in effect during the social periods. Social contingencies occurred on days 5 through 7 under active marijuana conditions and on days 11 through 13 under placebo conditions.

<ins>Drug administration</ins>. Marijuana cigarettes with 0% (placebo) and 1.84% delta-^{9}-THC concentrations were provided by the National Institute on Drug Abuse. Five puffs of each cigarette were smoked using a uniform puff procedure. Subjects smoked placebo or active marijuana cigarettes in their individual rooms at 10:00 and 15:25 and together in the social area at 19:25 and 22:00.

RESULTS

This analysis compares active marijuana administration (days 5-7) and the effects of placebo administration (days 11-13) on contingency controlled behavior. Data were analyzed relative to the baseline period occurring prior to each contingency. Under baseline conditions subjects spent between 1% and 32% of the social access period "reading", an activity done individually in each subject's private room. This activity functioned as the instrumental activity under contingency conditions for all subjects. Under baseline conditions, subjects spent between 41% and 76% of the social access period engaged in activities in the social area. Access to the social area served as the contingent activity during the contingency periods.

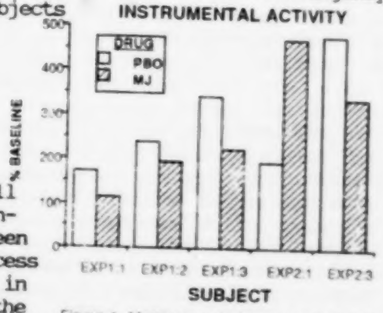

Figure 1. Mean percent change from baseline for time spent in the instrumental activity following placebo and marijuana administration during contingency periods for each of five subjects.

Figure I shows the increases in time spent engaged in the instrumental activity during the contingency periods as a percentage of non-contingent baseline time for 5 of the 6 subjects. The sixth subject's baseline time in the instrumental reading activity was less than 2% of the social access period and a meaningful contingency ratio could not be calculated. For all five of the remaining subjects however, instrumental activity increases ranged between 100% and 500% of baseline and 4 of the 5 subjects showed consistently greater increases in instrumental activity under placebo than under active marijuana conditions. Decreases in percent time spent in the contingent activity were observed during contingency periods, but there were no differences between the active marijuana and placebo conditions in this regard.

DISCUSSION

The results of this experiment extend the analysis of interactions between smoked marijuana and behavior to contingency-controlled social behavior. Increases in an instrumental performance maintained by access to social activity were smaller after active marijuana administration than after placebo administration. Time spent in contingent social interactions however, was decreased to a similar extent following both placebo and active marijuana administration. These results contrast the earlier reported effects of smoked active marijuana on behavior involving non-social assigned work tasks (Foltin et al., 1986). Under such private work conditions, there were no differences in the effects of active marijuana and placebo on increases in instrumental performance, but decreases in contingent activity were greater following active marijuana administration. Clearly, there are interactions between smoked marijuana and behavior that may vary with current contingencies and the functional properties of the performances observed.

Several early clinical reports suggested that repeated marijuana smoking may result in the loss of desire to work and an "amotivational syndrome" (McGlothin & West, 1968; Smith, 1968). For the most part, experimental studies of marijuana effects on operant performances maintained by monetary gain have provided little support for such "amotinational" phenomena (see review by Miles, 1975). The present findings however, do indicate that operant performances maintained by access to a preferred social activity may be, to some extent, attenuated following active marijuana administration. The relationship between the instrumental performance decrement following marijuana administration observed in the present study and the "amotivational syndrome" reported in clinical settings (e.g., Lantner, 1982; Voth, 1982) remains unclear. The results described in this report however, may provide the basis for an experimental analysis of the factors upon which such clinical observations are based, as well as those that account for the wide variability in marijuana effects described in the research literature.

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Nicotine Replacement Effects on Post-Cessation Withdrawal Symptoms and Weight Gain

M. Stitzer and J. Gross

Drug replacement therapy can be an effective tool in the treatment of substance abusers. A commercial chewing gum product (Nicorette) is available for the treatment of tobacco dependence that delivers nicotine via buccal absorption with 2 mg of nicotine contained in each piece of gum. Smokers are typically prescribed the gum just prior to cessation and are advised to chew up to 15 pieces per day for 3 months after quitting. Nicotine gum reduces the intensity of tobacco withdrawal symptoms following smoking cessation (Hughes et al. 1984; Schneider et al. 1984; West et al. 1984) and treatment evaluation studies have generally found that prescription of the gum results in increased cessation rates (Hughes and Miller, 1984). The rationale for 3-month gum prescription is not clear, however, since withdrawal symptoms may dissipate well before this time. Since the duration of post-cessation withdrawal effects is presently unknown, the first purpose of the present experiment was to study nicotine replacement effects on post-cessation withdrawal over a longer (10-week) time course than has previously been examined. Weight gain is a second common effect of smoking cessation (Hall et al. 1986; Wack and Rodin, 1982). Nicotine appears to exert a chronic suppressing effect on body weight that is released upon cessation of smoking (Wack and Rodin, 1982). Thus, there is reason to believe that post-cessation nicotine gum use might counteract the anticipated increase in body weight . Presently available data are inconclusive with regard to demonstrating that nicotine replacement can influence post-cessation weight gain (Fagerstrom, 1987). The second purpose of the present study was to examine post-cessation weight gain in people who remained smoking abstinent for 10 weeks while chewing under random assignment double-blind procedures a gum that contained 0 mg (placebo) or 2 mg nicotine.

METHODS

Subjects. Smokers were recruited from the community for a cessation program involving random assignment to active or placebo nicotine gum. One hundred twenty-seven attended an introductory meeting; 87 were assigned to a gum condition; 40 successfully completed the 10-week study. The 40 study completers were 55% female with a mean age of 42.7 years. They reported on average a 24 year smoking history and were smoking on average 30 cigarettes a day with a mean nicotine delivery of 0.8 mg nicotine. There was a significant between-group difference on reported cigarettes per day (placebo mean = 33, active mean = 25) but not on any objective measure of pre-cessation tobacco smoke exposure including expired air carbon monoxide, salivary cotinine or salivary thiocyanate.

Procedures. Research volunteers attended three pre-cessation smoking clinic meetings generally held on a Thursday, the following Monday and again on Thursday. They received a supply of their assigned gum under double blind conditions at the last Thursday meeting with the quit date set for the next Sunday. During the 10 week study, subjects reported to the laboratory twice weekly for data collection and were also visited at their homes for surprise breath sample collection, which provided an additional abstinence check. Home visits were conducted on two randomly selected days each week during the evening (7 - 10 pm) on weekdays and during the daytime on weekends. During laboratory visits, subjects were weighed, gave breath and saliva samples for analysis, turned in unused gum, received a fresh gum supply and filled out data questionnaires. A 15-item smoking withdrawal questionnaire was used that included all the DSM III criteria for tobacco withdrawal as well as some additional somatic complaints (Hughes and Hatsukami, 1986). Subjects rated the severity of each symptom on a 4-point scale from none to extreme. Subjects also rated the intensity of their craving for cigarettes on a four point scale from no cravings to severe cravings.

Study completion criteria. Subjects had to meet two requirements to successfully complete the study, 1) remain smoking abstinent for 10 weeks and 2) chew at least 5 pieces of gum daily throughout the study. Abstinence criteria were based on frequently collected expired breath samples and on salivary thiocyanate levels collected during post-cessation weeks 3, 6 and 9. Subjects were allowed up to 3 carbon monoxide readings during the entire 10 weeks that were above 9 ppm. A salivary thiocyanate level of 2500 umoles/liter was used as the cutoff indicating abstinence. The vast majority of the study disqualifications were because of smoking relapse; only 3 subjects were dropped because of noncompliance with the gum regimen.

Data analysis. Measures were collected once weekly or summarized over weekly intervals and analyzed in repeated measures analysis of variance or covariance for effects of treatment group, post-cessation time and group X time interaction. Baseline measures obtained two weeks prior to cessation were used as the covariate where appropriate. Measures analysed were: 1) gums used per day, 2) total withdrawal scale score, 3) scores on 15 individual withdrawal scale items and 4) body weight.

RESULTS

Gum use. Placebo gum study completers chewed an average of 5.7 pieces per day while active gum subjects chewed an average of 6.9 pieces per day; the difference was not statistically significant. However, a significant group X time interaction was observed. Both groups chewed an average of about 7 pieces per day during week 1. Active gum subjects remained at this level of use throughout the study while placebo subjects gradually decreased their use to an average of about 5 pieces per day (the required study minimum).

Withdrawal symptoms. Figure 1 shows average total withdrawal scores for 20 active and 20 placebo gum subjects over 10 successive post-cessation study weeks. It is clear that nicotine gum suppressed post-cessation reports of withdrawal symptoms (group effect F (1,37) = 10.6, $p < .005$). Both the time factor (F (9, 342) = 13.9, $p < .001$) and the group X time interaction (F (9, 342) = 2.5, $p < .03$) were significant. Average withdrawal scores for the active gum group decreased steadily from their pre-cessation baseline levels while scores for the placebo gum group increased immediately after cessation then declined.

Withdrawal item analysis. Two distinct time course patterns were apparent among the individual withdrawal items. Scores on four items: irritability, anxiety, impatience and difficulty concentrating, were lower in the active than in the placebo gum group during the first 4 - 5 post-cessation weeks but scores for the two groups had declined to a similar stable level by post-cessation week 6 (significant group X time interaction). In contrast, scores on five items: sweating, drowsiness, restlessness, excessive hunger and increased eating and also scores on the craving intensity question were significantly reduced in the active gum group throughout the 10-week post-cessation period. Items unaffected by nicotine gum condition were heart racing, dizziness, tremor, headaches, bowel/stomach problems and sleep disturbance.

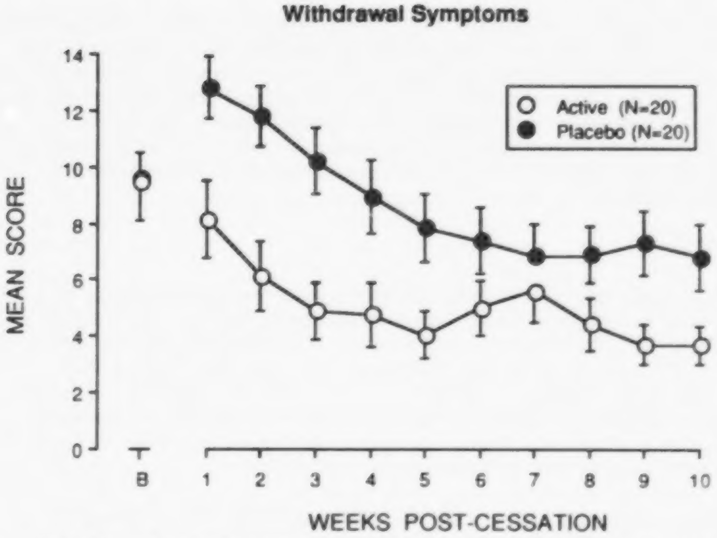

Fig. 1. Withdrawal symptom total scores (mean ± sem) are shown during baseline assessment (2-weeks prior to quitting) and over 10 post-cessation weeks for active gum (open circles; N = 20) and placebo gum (closed circles; N = 20) subjects. Subjects rated 15 items weekly on a scale of 0 = none to 4 = severe. Item scores were summed to obtain a total score.

Weight gain. Consistent with reports of less hunger and eating among active gum subjects, the study provided strong evidence for a gum effect on post-cessation weight gain (group effects F (1,37) = 10.6, p < .003). By week 10 placebo gum subjects had gained an average of about 8 pounds, while active gum subjects gained only about 4 pounds on average. These data suggest that using active gum resulted in a 50% reduction in cessation-related weight gain. The study also provided suggestive evidence of a nicotine dose-effect on weight gain. Active gum subjects who chewed conservatively (low use: mean = 5.4 pieces per day; N = 12) gained an average of 5 pounds while those who chewed more enthusiastically (high use: mean = 9.2 pieces per day; N = 8) gained 1.5 pounds. Weight gain suppression was most readily apparent at higher doses of active gum.

DISCUSSION

This study replicated previous findings by showing that tobacco withdrawal symptoms are suppressed during nicotine replacement therapy and extended previous reports by examining symptom patterns over a prolonged 10-week post-cessation period. Nicotine replacement suppressed total withdrawal scores throughout the 10 week post-cessation assessment, providing support for the typical 12 week duration of suggested post-cessation gum use. The item analysis time course data suggested, however, that many of the most disturbing symptoms of withdrawal (i.e. irritability, anxiety) dissipate by post-cessation week 6, after which nicotine replacement no longer exerts beneficial effects. Effects of nicotine replacement beyond 5 weeks were seen on reports of craving for cigarettes, excessive hunger and increased eating. The clinical significance of reduced cigarette cravings is not clear since relapse was not an outcome measure in this study. Further, our data are discrepant with previous studies showing no nicotine gum effect on post-cessation cigarette cravings or urges to smoke (West, 1984).

Consistent with subjective reports of reduced eating and hunger in active gum subjects, the study also showed that nicotine replacement can suppress the weight gain that is commonly seen after smoking cessation. The failure of previous studies to support a nicotine weight suppression hypothesis may be due to methodological weaknesses such as failure to verify gum use or to take amount and duration of gum use into account. The present study utilized sensitive methodologies to examine gum effects on post-cessation weight gain including continuous verification of both abstinence and gum use. The observation that weight gain was suppressed to a greater extent in those who chewed more gum is consistent with data presented by Fagerstrom (1987) and suggests a dose-effect relationship. However, the finding must be replicated in a study where dose is controlled rather than self-selected. Weight gain suppression may have important clinical implications. Weight gain is often cited as an undesirable effect of cessation and a reason for resuming smoking. If smokers can be motivated to use nicotine gum regulary in order to avoid weight gain, this could result in improved withdrawal suppression and cessation success.

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Efficacy of Nicotine Gum in General Practice: One Year Follow-up

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D. Ramlet, M. Healy and S. Higgins

Smokers (n=315) seen at a family practice and who wished to quit smoking were randomly assigned in a double-blind manner to either nicotine or placebo gum. Smokers received 10 minutes of physician advice, 10 minutes of nurse advice, 2 booklets, a 13 minute slide/tape show and a 10 minute followup.

At 1 year followup, 15% of those who received nicotine gum and 13% of those who recieved placebo gum were nonsmokers: i.e., reported continuously not smoking, had observers who verified this, had CO levels < 10 ppm, had SCN levels < 100 ng/ml and, if on placebo gum or not chewing nicotine gum, had salivary cotinines of < 10 ng/ml (p > .10). Similar results were obtained when only dependent smokers were examined.

These negative results replicate those of the two prior placebo-controlled trials conducted in medical practices (British Thoracic Society, Br Med J, 286:595, 1983; Jamrozik et al, Br Med J, 289:794, 1981). We hypothesize more motivated subjects and more intensive psychological therapy and followup are necessary for nicotine gum to be more effective than placebo gum.

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Effects of Secobarbital on Aggressive and Non-Aggressive Responding of Normal Male Subjects

D. Cherek, C. Sebastian and J. Steinberg

The self-administration of relatively large doses of secobarbital has been associated with an increased incidence of human aggressive behavior (e.g., Tinklenberg and Stillman, 1970; Tinklenberg, et al., 1974;1976). More recent clinical reports have observed increased probabilities of human aggressive behavior following barbiturate self-administration (Mayfield, 1983) and during barbiturate withdrawal (Kramer, 1983). This experiment assessed the effects of the acute administration of secobarbital on human aggressive and non-aggressive responding in a laboratory setting.

METHOD

Subjects

Twelve males have participated after giving their informed consent. Subjects were recruited by advertisements soliciting participation in behavioral research projects. The advertisements and consent form did not refer to aggressive behavior, since we did not want to imply that the research subjects must respond aggressively to participate in the experiment or to earn monetary payments.

All subjects were given a complete physical exam and structured psychiatric exam prior to drug administration. To avoid problems associated with drug usage, daily breath alcohol measures were taken and urine samples were obtained for complete drug screen analysis.

PROCEDURE

Subjects were told that they would be randomly paired with other people participating in the research project at the same time, but in different locations. The situation was described as one in which they could influence the amount of money earned by these other individuals by subtracting money from them. Subjects were told that the people with whom they were paired could choose to subtract money from them at any time during the experimental sessions. Subjects were also informed that money subtracted from them would be added to the other person's earnings. However, money they subtracted from the other people would not be added to their earnings.

Five subjects participated in a time-course study and came into the medical center once per week. These subjects participated in five successive 30 minute sessions conducted from 8:00 AM to 1:00 PM. Sessions were begun 0.5, 1, 2, 3 and 4 hours after drug administration. The other subjects were in the dose-response studies and came into the medical center for daily 50 minute sessions five days per week (Mon. through Friday).

Subjects were required to swallow two #00 gelatin capsules 60 minutes prior to the sessions. These capsules contained either placebo or 50, 100 or 200 mg of secobarbital per 70 kg of body weight. Successive doses were separated by at least 72 hours, and were administered if preceding placebo session responding was within variability ranges established prior to drug administration.

The response console contained two response manipulanda, response button A and B. Pressing button A was maintained by a fixed-ratio (FR) 100 schedule of point presentation. Each point delivery was indicated by incrementation of a counter mounted directly adjacent to button A and was equivalent to ten cents. Pressing button B ostensibly delivered an aversive stimulus to another person and was defined as aggressive. The completion of each fixed-ratio (FR) 10 on button B resulted in the ostensible subtraction of one point, i.e., ten cents, from the other person.

Aggressive responding was established by subtracting money from the subjects, which was attributed to the other participants. Point subtractions (provocations) were scheduled to occur at random points throughout the session. In the absence of aggressive responding, subjects were scheduled to receive 40 provocations (point subtractions) per session. In addition to ostensibly subtracting a point from the other person, ten responses on button B also initiated a provocation-free interval (PFI) during which point subtractions were not presented. PFI durations were either 125 or 500 seconds. Subjects were able to initiate a PFI only following at least one point subtraction, i.e., an escape contingency. When the PFI elapsed, point subtractions were again presented randomly.

Subjects completed the Profile of Mood States (POMS) questionnaire and were evaluated for clinical signs of intoxication after each session. Subjects also completed the BUss-Durkee Hostility Inventory at the end of the study. Following their participation, subjects completed a series of questionnaires to determine if the subjects believed they were paired with other people and then they were debriefed.

RESULTS

The time-course study of the effects of secobarbital on aggressive responding 0.5, 1, 2, 3 and 4 hours after drug administration are shown in figure 1. Four of the five subjects increased aggressive responding following the administration of secobarbital. Increased aggressive responding was observed at 0.5, 1 and 2 hours after secobarbital administration. Subject S-209 had a large increase in aggressive responding at the lowest dose (50 mg per 70 kg), and the higher doses either decreased aggressive responding (100 mg per 70 kg) or completely eliminated responding (200 mg per 70 kg). This subject previously participated in a diazepam study, in which diazepam (10 mg per 70 kg) produced substantial decreases in aggressive responding. Subject S-231 decreased aggressive responding, particularly 1 hour after the 200mg per 70 kg secobarbital dose. At the end of participation, questionnaires indicated that this subject did not

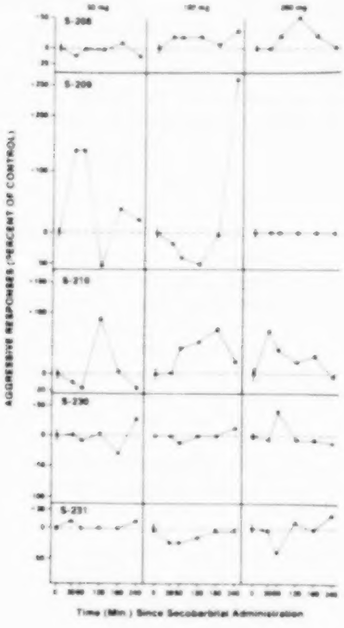

FIGURE 1. The effects of placebo and three doses of secobarbital (50, 100 and 200 mg per 70 kg of body weight) on aggressive responding 0.5, 1, 2, 3 and 4 hours after drug administration. Data points are expressed as percentage changes from the preceding placebo sessions. All subjects were assigned to PFI durations of 125 seconds.

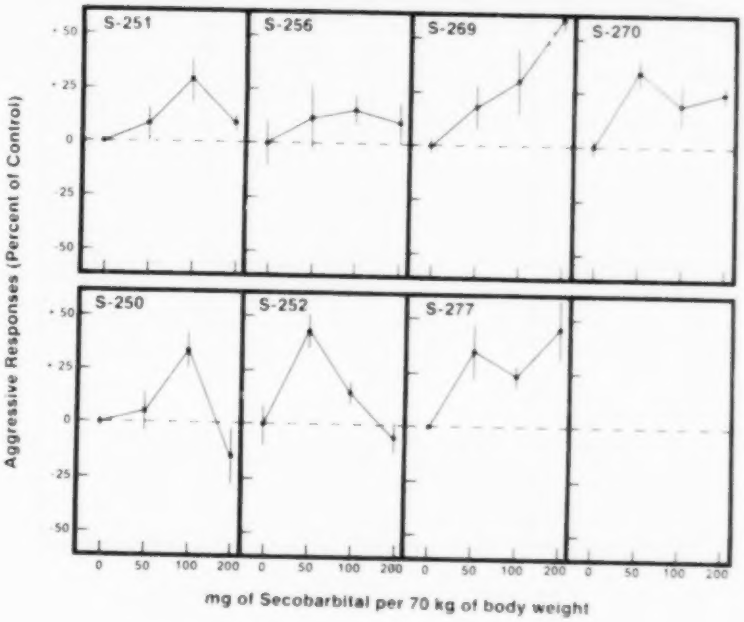

FIGURE 2. The effects of placebo and three doses of secobarbital (50, 100 and 200 mg per 70 kg of body weight) on aggressive responding. Data points are expressed as percentage changes from the placebo session values set at zero. Drug data points represent the mean of three different sessions. Vertical lines at all data points represent ± SEM. Subjects assigned to PFI durations of 500 seconds are shown in the top half of the figure, and those assigned to PFI durations of 125 seconds are shown in the bottom half of the figure.

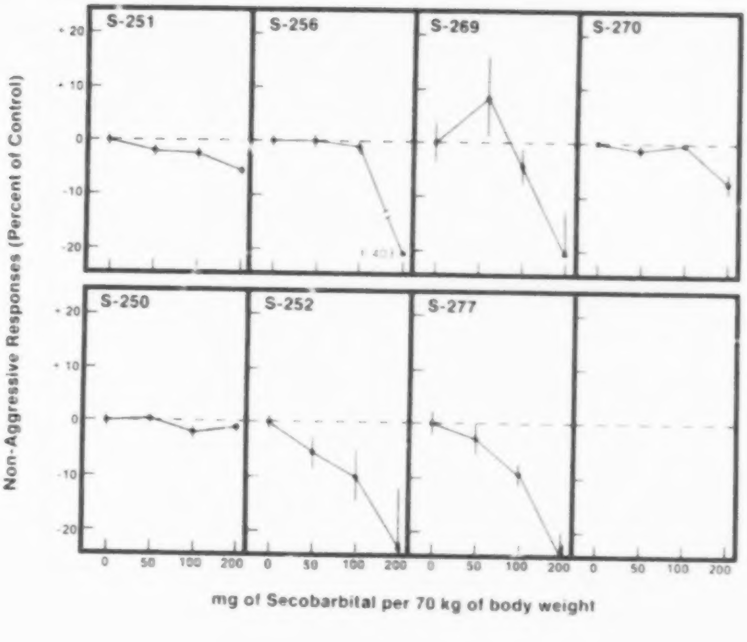

FIGURE 3. The effects of placebo and three doses of secobarbital (50, 100 and 200 mg per 70 kg of body weight) on non-aggressive responding. Data points are expressed as percentage changes from the placebo session values set at zero. Drug data points represent the mean of three different sessions. Vertical lines at all data points represent ± SEM. Subjects assigned to PFI durations of 500 seconds are shown in the top half of the figure, and those assigned to PFI durations of 125 seconds are shown in the bottom half of the figure.

accept the cover story and felt that a machine was subtracting his points rather than another person. All other subjects indicated that they believed they were paired with other people. It is interesting to note, that the one subject that indicated he did not accept the cover story was the only subject that decreased aggressive responding following secobarbital administration. Non-aggressive responding was decreased following secobarbital administration at the 100 and 200 mg per 70 kg doses. Peak decreases were typically observed at 1 and 2 hours after drug administration.

The effects of placebo and three doses of secobarbital (50, 100 and 200mg per 70 kg) on aggressive responding are shown in Figure 2, for subjects participating in dose-response studies. All subjects increased aggressive responding following secobarbital administration. Two subjects had the largest increase in aggressive responding following the 50mg per 70kg dose, while three subjects had maximal increases at 100mg, and two subjects had largest increases at the highest secobarbital dose. A repeated measures ANOVA analysis indicated that the effect of secobarbital dose was significant (F=3.12,d.f.=3,18 p<.05).

The effects of placebo and three doses of secobarbital on non-aggressive monetary reinforced responding are shown in Figure 3. All subjects decreased non-aggressive responding following secobarbital administration. Decreases in non-aggressive responding were dose-dependent, with the largest decreases observed at the highest secobarbital dose. A repeated measures ANOVA analysis indicated that the effect of secobarbital dose was significant (F=7.64,d.f.=3,18 p<.001).

DISCUSSION

The acute administration of secobarbital increased aggressive responding in normal male subjects in a laboratory setting. This effect is similar to reports of violence associated with secobarbital self-administration among young adolescent males. The increased aggressive responding following secobarbital administration appears to be specific, since simultaneously occurring non-aggressive responding was decreased. Increased aggressive responding following secobarbital administration was the result of subjects engaging in more frequent aggressive responding following provocation and occasionally initiating aggressive responses in the immediate absence of provocation. Alcohol was also observed to increase aggressive responding in some subjects (Cherek et al., 1985), but the effects of secobarbital produced consistent increases in aggressive responding in all subjects.

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Hyperprolactinemia During Cocaine Withdrawal

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and A. Skupny

ABSTRACT

Plasma prolactin levels were determined for 8 patients (7 males and 1 female) who reported an average of 3.7 years of cocaine abuse. The mean prolactin level for these 8 patients 24 to 72 hours following admission to the hospital was 20.58 (S.E. ± 3.2) ng/ml. Hyperprolactinemia ($>$ 20 ng/ml) was detected in 4 patients and 3 others had high borderline prolactin levels. Only 1 patient had a normal prolactin value at the time of admission to the hospital. The mean prolactin level obtained prior to the patients' discharge from the hospital was 31.7 (S.E. ± 5.3) ng/ml. Five of the 8 patients had an increase in prolactin levels between time of admission and discharge from the hospital. The mean increment in plasma prolactin levels for these 5 patients was 15.5 (S.E. ± 4.2) ng/ml ($P < .02$). Two patients who had significant hyperprolactinemia ($>$ 50 ng/ml) at the time of discharge from the hospital reported self-administering the highest cocaine dose (6-8 gm per week) of the 8 patient cohort. Since cocaine blocks dopamine reuptake and prolactin secretion is regulated, in part, by dopaminergic inhibitory mechanisms, cocaine withdrawal may induce rebound supersensitivity of prolactin secretion. Findings obtained in this study suggest that cocaine-related derangements in prolactin secretion may be a biologic marker of a protracted cocaine abstinence syndrome.

INTRODUCTION

The cocaine epidemic in the United States during the past decade has involved widespread use of the drug by both men and women (Kozel and Adams 1986). Most women who abuse cocaine are of childbearing age (Washton *et al.*, 1985). Although cocaine self-administration is purported to initially enhance libidinal drive (Siegel 1984), recent clinical studies indicate that chronic cocaine abuse is associated with decreased libido and sexual dysfunction (Washton *et al.*, 1985). In males, impotence (Ashley 1975) and gynecomastia have been observed in regular cocaine users, and these abnormalities have persisted for long periods following drug abstinence (Cocores et al., 1986). Women who abuse cocaine have reported major derangements in menstrual cycle function including galactorrhea (Cocores et al., 1986), amenorrhea and infertility (Siegel 1982).

There are also recent reports that cocaine abuse may adversely effect pregnancy. In 1985, Chasnoff and his associates reported that "the number of cocaine-using pregnant women presenting to the perinatal addiction project of Northwestern Memorial Hospital has escalated dramatically in the past 2 years." These investigators studied 23 infants who were born to cocaine-using women and concluded that "cocaine exerts an influence on pregnancy outcome as well as neonatal behavior." Chasnoff and his associates also stated that there is also the possibility that cocaine exposed infants are "at risk for a higher rate of congenital malformations and perinatal mortality." It therefore appears that cocaine abuse, like alcohol, cannabis and opiate abuse by pregnant women, significantly enhances risk for abnormalities of growth and development of the newborn.

The mechanisms by which cocaine adversely effects reproductive function and fetal development are unknown and there have been few systematic studies of endocrine function in cocaine abusers. However, recent clinical reports suggest that cocaine abuse may be associated with alterations in prolactin levels (Cocores et al., 1986, Dackis and Gold 1985, Gawin and Kleber 1985). One study (Gawin and Kleber 1985) reported a cocaine-related decrease in plasma prolactin levels of men and women. However, a second study (Dackis and Gold 1985) found that prolactin levels were significantly greater in men who abused cocaine in comparison to age-matched normal controls. A third study (Cocores et al., 1986) reported hyperprolactinemia and sexual dysfunction in 7 of 10 chronic cocaine abusers. The purpose of this study was to determine plasma prolactin levels in men and women admitted to the McLean Hospital for treatment of cocaine abuse during early and protracted cocaine abstinence.

METHODS

Eight patients admitted to the Alcohol and Drug Abuse Treatment Center of the McLean Hospital for treatment of cocaine abuse participated in this study. All patients were caucasian, 7 were males, 1 was a female. Their mean age was 27.9 years (range 20-38 years). Patients reported a history of cocaine abuse averaging 3.7 years (range 2-10 years). All patients self-administered cocaine via the intranasal (snorting) route. The amount of cocaine regularly self-administered ranged from 1 1/2 to 8 grams per week.

All patients reported withdrawal symptoms during the first 24 to 48 hours following admission to the hospital. These symptoms included headache, backache, anxiety, insomnia, and persistent craving for cocaine.

A plasma sample for prolactin analysis was obtained 24 to 72 hours following admission after reports of withdrawal symptoms had abated. At this time no patient had any intercurrent medical disorder; physicial examinations including blood pressure and cardiovascular function were normal and all routine blood chemistry and hemogram studies were within normal ranges. A second blood specimen was obtained for prolactin analysis immediately prior to the patient's discharge from the hospital (mean interval between samples was 22 days).

Prolactin concentrations were measured in duplicate plasma samples using a double antibody radioimmunoassay procedure similar to that described by Midgley (1966), with materials provided by the National Hormone and Pituitary Program (University of Maryland School of Medicine), supported by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (NIADDK).

The prolactin assay sensitivity is less than 3.1 ng/ml. Intra- and interassay coefficients of variation were 6.8 and 14 percent, respectively.

RESULTS

Figure 1 shows plasma prolactin levels (ng/ml) for the 8 patients following admission to the hospital (sample 1) and prior to discharge (sample 2). The mean prolactin value after admission was 25.8 (± 3.2) ng/ml and mean prolactin value for sample 2 was 31.7 (± 5.3) ng/ml. Hyperprolactinemia (> 20 ng/ml) was observed in 4 patients (numbers 1,3,5, and 7) shortly following admission to the hospital. Three other patients (numbers 2,6,8) had high borderline prolactin levels. Only 1 patient (number 4) had a normal prolactin level at the time of admission to the hospital.

Only 2 patients had normal prolactin levels (< .20 ng/ml) at the time of discharge from the hospital. Five of the 8 patients (numbers 1,2,3,4,8) showed an increase in plasma prolactin levels prior to discharge (sample 2). The mean increment in plasma prolactin for these 5 patients was 15.5 (± 4.2) ng/ml) and was statistically significantly different ($P < .02$) from mean prolactin levels following admission to the hospital. Two patients had a decrease in plasma prolactin levels (numbers 5,7). However, 1 of these patients (number 7) continued to have an elevation of prolactin levels (35 ng/ml) which was in the hyperprolactinemic range. One patient (number 6) had a slight decrease in borderline high plasma prolactin values.

Two patients (numbers 1 [female], and 3 [male]) who had abnormal prolactin levels following admission to the hospital showed significant hyperprolactinemia (> 50 ng/ml) at the time of discharge. It is of interest that these 2 patients reported self-administering the highest cocaine dose (6-8 grams per week) of this 8 patient cohort.

DISCUSSION

These data are consistent with previous reports (Cocores et al., 1986, Dackis and Gold 1985). In 1985, Dackis and Gold reported studies of serum prolactin levels in 18 male cocaine abusers and 20 normal age-matched male controls. Prolactin levels obtained in studies with the cocaine abusing patients (35.4 ± 26.9 ng/ml) were significantly greater than those of the control subjects (7.0 ± 5.0 ng/ml). Dackis and Gold (1985), interpreted these findings as "consistent with either decreased functional dopaminergic tone or activation by cocaine of serotinergic transmission." Dackis and Gold (1985), studied cocaine abusers during inpatient hospitalization, and thus their data were less likely to be confounded by factors associated with studies carried out with outpatient populations. Cocores and co-workers (1986), also reported hyperprolactinemia in patients during cocaine withdrawal.

However, these data are not consistent with reports of studies with an outpatient population. Gawin and Kleber (1985), measured plasma prolactin levels in 15 males and 6 females who abused cocaine for an average of 3.4 years. Female plasma prolactin levels were within the range of normal values for adult women, but plasma prolactin levels were decreased in male cocaine abusers when compared with healthy age-matched control subjects. Although the mean plasma prolactin levels for male cocaine abusers was 5.3 ng/ml, i.e. within the range of normal prolactin levels for healthy adult males, 5 of the 14 males who abused cocaine had abnormally low prolactin levels.

Gawin and Kleber (1985) interpreted these findings cautiously, noting that "the methodological shortcomings of the research on recently active drug users preclude firm conclusions being reached." They pointed out that their patients exhibited marked heterogeneity which included "differences in the purity of cocaine obtained, chronicity of use, routes of administration, self-report accuracy, sensitivity to cocaine effects, and degrees of dependence. Other shortcomings of outpatient clinical studies which are unrelated to cocaine abuse itself, such as possible undetected use of other drugs or medication, differences in diurnal patterns, sleep habits, diet, or psychosocial stressors and the possibility of prexisting endocrinopathy could have also effected the results." Because the patients studied by Gawin and Kleber (1985) were recruited from an outpatient clinic population, it was not possible to control for many critical factors which effect prolactin secretion.

Our studies, as well as the studies of Dackis and Gold (1985) and Cocores et al., (1986), indicate that chronic cocaine abuse may lead to significant derangements in prolactin secretory activity. Moreover, our findings suggest that chronic cocaine abuse may lead to prolonged derangements of anterior pituitary function, even during protracted cocaine abstinence.

There is considerable evidence that cocaine may block dopamine reuptake (Iversen 1966, Reichlin 1975, Ross and Renyi 1966, Scheel-Kruger 1971, Taylor and Ho 1978, Trendelenburg and Graefe 1975, Whitby *et al.*, 1960), and it is also known that dopaminergic systems are important modulators of prolactin secretion (Tuomisto and Mannisto 1985). Since prolactin secretion in humans is under chronic dopaminergic inhibitory control, it may be postulated that cocaine administration would suppress prolactin levels. However, following cocaine withdrawal, it would also be reasonable to postulate that rebound supersensitivity of prolactin secretion would occur. The elevated prolactin levels found in this study, as well as the elevation in prolactin levels reported by Dackis and Gold (1985) and Cocores *et al.*, (1986), are consistent with this hypothesis. However, the continued elevation in prolactin levels found in 5 of our 8 patients during protracted cocaine abstinence was surprising. This observation suggests that a cocaine-related derangement in dopaminergic regulatory systems may parallel a protracted cocaine abstinence syndrome. Since a protracted cocaine abstinence syndrome may be an important factor which effects recrudescence of cocaine use, plasma prolactin levels may be a useful biologic marker for determining relapse potential.

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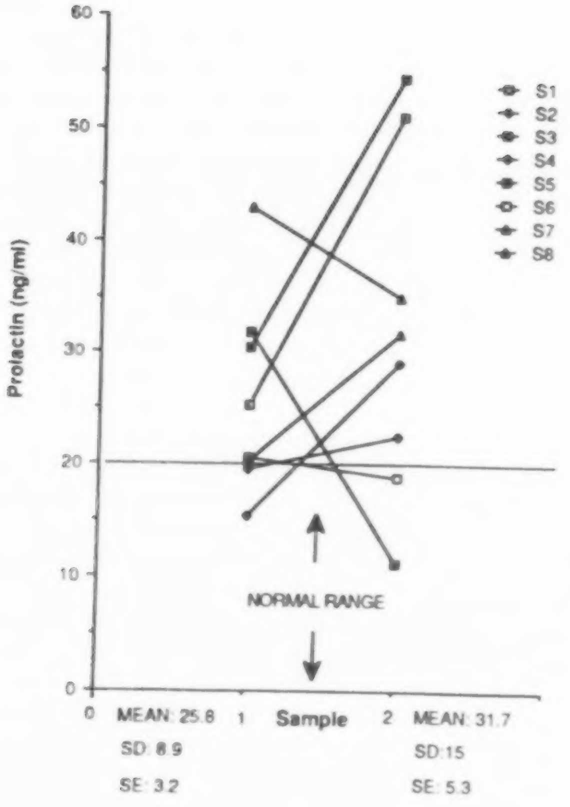

FIGURE 1

Plasma prolactin levels (ng/ml) for 8 patients (s1 - s8) shortly following admission to the hospital (sample 1) and immediately prior to discharge (sample 2).

Conditioned Craving and Arousal in Cocaine Addiction: A Preliminary Report

A. Childress, R. Ehrman, A. T. McLellan and C. O'Brien

INTRODUCTION

For several years our research group has studied the conditioned responses associated with chronic ***opioid*** use, speculating that some of these responses (particularly conditioned craving and withdrawal) could lead to drug use and relapse in the abstinent patient (O'Brien et al., 1977, Childress et al., 1984, 1985, 1986). The recent upsurge of ***cocaine*** use among our patients has given us the opportunity to study and to document the kinds of conditioned responses which may occur in chronic cocaine abusers (Childress et al., 1987a).

A number of detoxified cocaine users in our clinic population report experiencing intense arousal and cocaine craving when they encounter 'reminders' of their previous cocaine use: the sight of cocaine-using friends or locations, the use of alcohol, the sight of white bread crumbs on the carpet, even the sight of talcum powder while changing a child's diaper. Apparently, almost *any* stimulus that has been *repeatedly* associated with getting and using cocaine can become a cocaine 'reminder' (Siegel, 1984; Washton, 1986).

In our view, these cocaine 'reminders' are essentially ***classically conditioned stimuli*** which have acquired their 'reminder power' through *repeated pairings* with cocaine's pharmacologic effects over the natural course of a patient's drug use. By giving repeated exposures to conditioned cocaine 'reminders' *without cocaine* , it should be possible to reduce or to ***extinquish*** their ability to trigger the conditioned responses (arousal, craving , etc.) which could lead to drug use and relapse.

At our research center we have recently begun a large-scale study attempting to understand and to treat the causes of ***relapse*** in cocaine dependence. As one part of this program we are trying 1) to ***characterize*** the ***kinds*** of responses patients may experience when they are exposed to cocaine 'reminders', 2) to ***measure*** these responses, and 3) to ***reduce*** these responses through ***extinction*** (repeated non-reinforced exposure).

METHODS

Subjects . The subjects of this initial report were 29 male Veterans presenting to the Philadelphia Drug Dependence Treatment Unit with a primary problem of cocaine dependence. The demographic and clinical characteristics of this population have been more fully described in a recent report (Childress, l987b). Of these, the first 9 patients were pilots; the second group of 20 patients were randomly-assigned participants in a large scale treatment-outcome study investigating the potential benefits of adding extinction (repeated exposure to cocaine 'reminders') and other interventions to standard drug treatment.

General Procedures

Laboratory Measurement Sessions. Prior to extinction or other treatments, all patients were tested for their initial responsivity to *cocaine-related* stimuli in a 90-minute laboratory measurement session. Both laboratory and extinction sessions have been described in detail in recent publications (Childress et al., l986, l987b) and will be summarized very briefly here. Both ***physiological*** and ***subjective*** measures were obtained. Physiological measures included peripheral skin temperature (**TEMP**), galvanic skin resistance (**GSR**), a general arousal index, heart rate (**HR**), and respiration (**RESP**). *Subjective* measures were obtained by asking each abstinent patient to rate, on a 1 to 10 scale, the degree of subjective ***cocaine high***, ***craving***, or '***crash***' (withdrawal) experienced under each set of stimulus conditions. The following stimulus components were used: 1) Neutral Baseline, 2) Neutral *Videotape* (a nature story), 3) Neutral *Activity* (video pong game), 4) Drug Baseline, 5) Drug-related *Videotape* (buy-sell and cocaine administration rituals), 6) Drug-related *Activity* (handling drug paraphernalia and performing a simulated cocaine administration) and 7) Recovery Baseline. To control for order effects, approximately half the patients experienced the stimuli in the order listed above ('Forward' condition), while the remaining patients experienced the Drug-related video/activity *before* the Neutral video/activity

('Reverse' condition).

Extinction Sessions. Patients assigned to extinction groups received 15 hour-long sessions of repeated, non-reinforced exposure to cocaine 'reminders'. Each hour-long cocaine extinction session contained 3-five minute *audiotape* segments, 3 five-minute exposures to a cocaine-related *videotape* and 3 simulated cocaine administration rituals. These drug-related stimuli were presented in the sequence *audio-video-activity*, repeated 3 times per session.

For both laboratory and hospital ward extinction sessions, data reported here derive from patient ratings of the *overall* intensity of cocaine-like high, craving, and 'crash' (withdrawal) using a 1-10 scale for each rating. These items are part of the Within-Session Rating Scale-Cocaine(l985), which was administered at the *beginning* and again at *the end* of each extinction session.

RESULTS

In the interest of conserving space, the reader is referred to recent reports (Childress et al., l987a; l987b) for detailed derivation of the percentage baseline values (physiological measures) or difference scores (subjective measures) used in the analyses below.

Pretreatment Laboratory Testing

Physiological Measures (n=17). Analyses first were completed for **GSR** and **TEMP**, as these physiological variables have historically best reflected the conditioned arousal associated with drug-related stimuli. A three-way ANOVA revealed significant main effects of Type (**Drug vs. Neutral**, $p <. 003$), Mode (**Video vs. Activity**, $p<.003$), and Order of stimulus presentation (**Forward vs. Reverse**, $p<.003$) on **TEMP,** with no significant interactions among these three variables. Thus, as shown in Figure 1, skin temperature reductions were significantly greater to *Drug (cocaine)-related* than to *Neutral* stimuli; and were generally greater in response to activities than to video stimuli. The Forward order of stimulus presentation (Neutral stimuli first, Drug-related stimuli second) resulted in greater overall temperature reductions, suggesting that patients are more aroused during the Neutral stimuli *when they are anticipating the Drug-related stimuli*, and that this general arousal sums with the drug-related arousal to produce a larger temperature response.

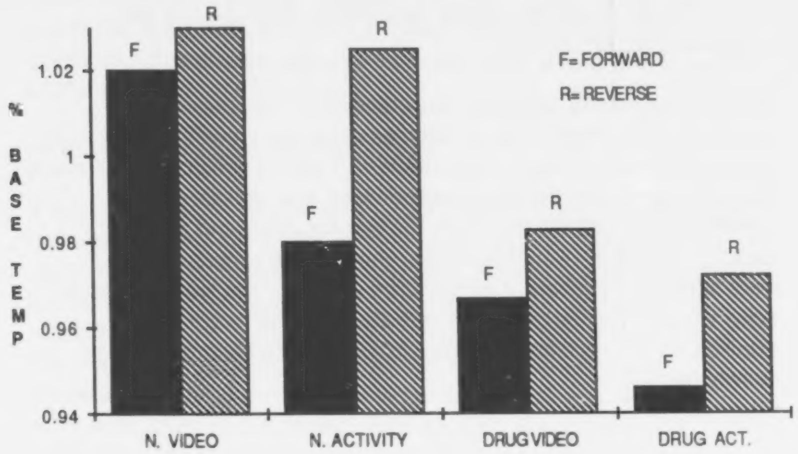

Figure 1. *Skin temperature response in cocaine abusers (N =17)*

The *average* temperature reduction to cocaine- related stimuli (including 'non-responders') was approximately 4 Fahrenheit degrees. Among differential temperature 'responders', however, dramatic reductions of 8 to 12 Fahrenheit degrees (in response to cocaine-related stimuli) were not uncommon. The magnitude and pattern of these temperature changes in response to **cocaine-related** stimuli is *similar* to the changes which occur in response to **opioid-related** stimuli, frequently reported by our laboratory (Childress et al., 1985,1986).

For the physiological variable of **GSR** (skin resistance), the three-way ANOVA revealed a similar pattern of results: there was a main effect of both stimulus Type (**Neutral** vs. **Drug-related**, p<.001) and Mode (**Video** vs. **Activity,** P<.000). There was a significant Type X Mode interaction effect(P<.01) : **GSR** values were significantly different for Neutral vs. Drug-related **Video** stimuli, but did not differ significantly between Neutral and Drug-related **Activities**. There was no significant effect of Order (**Reverse** vs. **Forward**) of stimulus presentation upon **GSR** (p>.15).

Subjective Responses (N=21) An overall ANOVA (with stimulus condition as the repeated measure) was performed upon each of the

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subjective variables of self-rated ***high, craving***, and ***withdrawal*** . These overall analyses revealed a significant effect of stimulus condition upon cocaine *craving* (p<0.0000), *high* (p<.01) and *withdrawal/'crash'* (p<.01).

Of these responses, ***craving*** was clearly the most prevalent, reported two to three times as often as either high or withdrawal/ 'crash' responses. Subsequent Bonferroni paired comparisons showed that *craving* to both the Drug-related video *and* activity differed significantly from craving to *both* the Neutral video and activity (p<.01). Additional Bonferroni tests showed that reports of *high* were significantly different for the Drug *video* vs. the Neutral task or activity (p<.05) ,while reports of *withdrawal/'crash'* were significantly different for the Drug *activity* vs. the Neutral video or activity (p<.05).

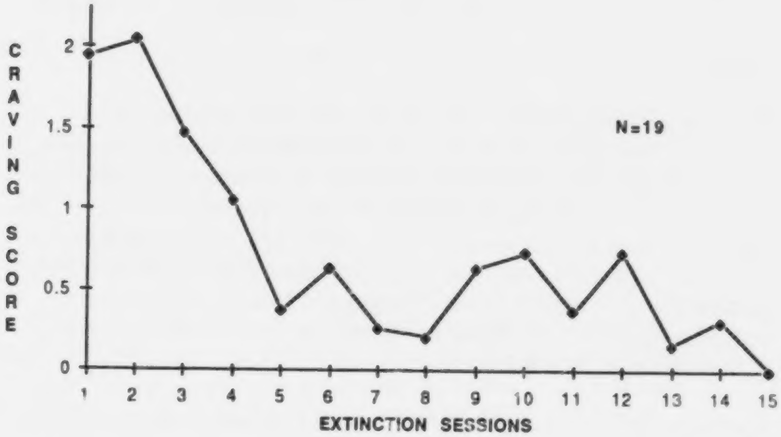

Figure 2. *Reduction in cocaine craving as a function of extinction .*

Extinction Sessions

Subjective Responses. A one-way ANOVA with repeated measures was performed for each of the subjective variables of ***craving***, ***high***, and ***withdrawal/'crash***', using sessions as the repeated measure. These analyses revealed a significant effect of sessions upon all three subjective variables: craving (p<.0000), high (p<.0001), and withdrawal 'crash' (p<.0000). Of these responses, craving was the most prevalent and persistent, reducing gradually over the course of fifteen extinction sessions. **Figure 2** (above) shows the reduction in craving as a function of extinction trials. Reports of high and 'crash'/withdrawal were less common, and were largely extinquished by the sixth hour of extinction.

PRELIMINARY CLINICAL OUTCOME

A few early indicators of clinical outcome are available. Thus far, all patients who entered the study have been retained throughout the two week inpatient treatment phase and are generally compliant with the intensive extinction regimen. Despite this regimen, *physiological* arousal to cocaine 'reminders' was still evident (though reduced) at the end of the inpatient stay for more than half the extinction patients. Though *craving* to the *extinction stimuli* was virtually eliminated, most patients have reported episodes of craving in response to *real-world* cocaine 'reminders' during the two month period following hospital discharge. Given the incomplete extinction of physiological arousal and the incomplete *generalization* of extinction for subjective craving, it is perhaps not surprising that approximately two thirds of the patients have at least one episode of cocaine use during the two month period following the inpatient treatment phase.

SUMMARY

Though data collection is still in progress, several significant findings are already apparent from our study of cocaine 'reminders':

1) In the *laboratory*, detoxified cocaine abusers show a ***differential responsivity*** to drug-related cocaine 'reminders', responding with strong signs of ***physiological arousal*** (peripheral skin temperature reductions and decreases in skin resistance) and ***subjective*** cocaine ***craving.***

2) In *extinction* sessions, repeated, non-reinforced exposure to cocaine 'reminders' led to a ***complete reduction in craving*** to these stimuli by the fifteenth hour-long session. High and 'crash' responses were virtually eliminated by the sixth hour of extinction.

3) ***Physiological arousal*** to cocaine 'reminders' was often still in

evidence *even after* fifteen hours of extinction.

4) Even *after* completing the current extinction protocol cocaine abusers may crave--and use--cocaine when experiencing drug 'reminders' in the natural environment.

Clearly, detoxified cocaine abusers can experience conditioned craving and arousal to cocaine 'reminder' stimuli. These responses can be both intense and persistent, meaning that the abstinent cocaine abuser may be vulnerable long after detoxification is complete. Though the program of extinction described here is effective in reducing craving to cocaine-related stimuli presented *in the context of the laboratory or clinic*, this effect may not generalize well to the natural environment. We are currently considering two approaches to this problem: 1) One approach would attempt to *increase the generalization of extinction* by the use of even more realistic stimuli (e.g.,the sight of real cocaine) and stimulus contexts (e.g., *in vivo* repeated exposures). We have been reluctant to employ *in vivo* exposures near 'copping' corners or shooting galleries because of possible risk to both patients and clinical staff. Somewhat less dangerous stimuli could involve the patient's own home, or the use of 'neighborhood' videos taped from a moving car. 2) A second approach would *explore the effectiveness of other behavioral techniques* in countering conditioned craving and arousal. These techniques could include the training of *cognitive or behavioral alternatives* in response to cocaine 'reminders', encouraging the patient to actively cope with the arousal triggered by these stimuli. This second approach is clinically quite compatible with extinction and could conceivably enhance its effectiveness: patients could practice these coping techniques in response to the repeated, non-reinforced presentation of cocaine 'reminders'.

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A Pilot Trial of Amantadine for Ambulatory Withdrawal for Cocaine Dependence

C. Morgan, T. Kosten, F. Gawin and H. Kleber

INTRODUCTION

Recent focus on neurochemical adaptation in chronic cocaine abusers has increased interest in possible pharmacologic intervention in this population. Dopaminergic autoreceptor supersensitivity has been postulated to be the mechanism underlying post-cocaine dysphoria or craving and contribute to relapse. To deal with the early phase of cocaine withdrawal, two medications acting on the dopaminergic system have been proposed, bromocriptine and amantadine. Bromocriptine, a direct agonist has been shown to be more effective than placebo in reducing cocaine craving and other signs of withdrawal in single dose studies. In a 10 day double-blind comparison of amantadine and bromocriptine by Tennant and Sagherian, both drugs were effective, however, side effects of bromocriptine led to drop-outs.

This study examines the effect of amantadine on a) acute and short-term craving, b) cocaine use, and c) compliance with outpatient treatment.

SUBJECTS AND METHODS

Twelve patients aged 19 to 42 years old who had sought treatment for cocaine abuse at the Yale Substance Treatment Unit were included. All subjects met DSM-III criteria for cocaine abuse, and had used cocaine within ten (10) days. None were dependent by DSM-III criteria on other drugs or alcohol or met criteria for other Axis I diagnosis.

Patients gave informed consent after being explained the rationale for the use of amantadine for cocaine withdrawal, and asked to participate in a 30 day drug trial. For the first two days subjects entered a blinded cross-over, where they received identical looking capsules of either placebo or active drug Day 1 and then switched to the other form on Day 2. From Day 3 to the completion of treatment subjects were given prescriptions for amantadine hydrochloride 300 mgs daily for each week of study. Patients continued to be seen by their clinicians on a weekly basis and had weekly ratings.

Subjects were asked to keep track of their daily craving on a linear analog scale, and a narrative section and to report weekly the number of grams of cocaine used. Random urine monitoring for benzolyecognine was done during the drug trial period. On a weekly basis patients were also asked whether the medication affected their mood, energy level or sleep pattern. Side effects were monitored including blurred vision, insomnia, dry mouth, confusion, lightheadedness, anxiety, weakness, gastrointestinal symptoms. Finally patients who used cocaine during the study were asked to describe whether they perceived any change in the effects of cocaine while taking amantadine.

RESULTS

The majority of patients remained in the study for 14 or more days (10/12). Three remained for the entire length of the study.

Compared to baseline the mean craving scores decreased within 24 hours of starting amantadine. This was not statistically significant. Furthermore, patients on placebo experienced a similar decrease in craving over the same time period. (Figure 1). At 14 days mean craving scores had decreased to less than 50% of baseline ($p < 0.01$). (Figure 2)

Cocaine use at endpoint was decreased in the majority of patients (8/12) and two patients remained at their low level of baseline use. One patient dropped out after 6 days on medication and presumably resumed cocaine use and another increased to above baseline use at the time he left the study.

Individual patients experienced fluctuations in craving while on amantadine. In particular conditioned cues caused increased craving which often led to cocaine use. Conditioned cues included 1) anticipating and/or receiving paychecks, 2) telephone or other contact with drug dealers or users, 3) social settings where alcohol was used, 4) job tension and, 5) family arguments.

Five patients reported side effects attributable to amantadine (dizziness, tinnitus, drymouth, insomnia). Reduction in dosage from 300 to 200 mgs daily eliminated side effects in two patients and allowed them to continue treatment. In the others, side effects were time limited and did not adversely affect compliance with treatment.

None of the patients taking amantadine reported untoward effects with continued cocaine use. No qualitative difference in cocaine-induced euphoria was reported by any patient on amantadine.

While the majority of patients remained in the study through the 14th day, there was a 50% drop-out between the 14th and 23rd day (Figure 3). Two patients reported that the "medication didn't work" during the second or third week and the craving scale of one patient paralleled this. No explanation was obtained from the other drop-outs.

FIGURE 1

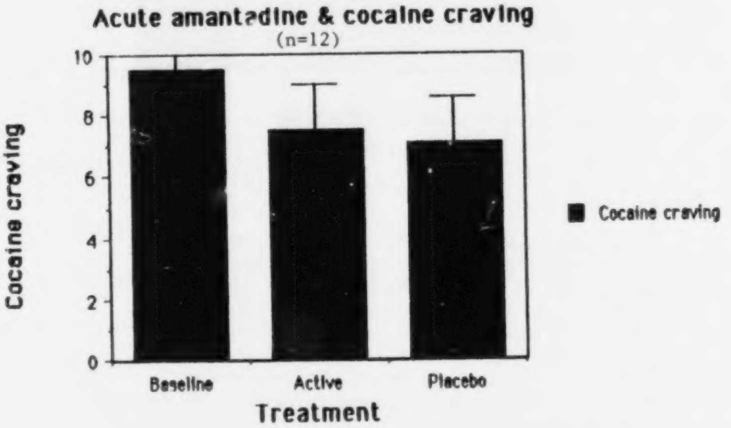


FIGURE 2

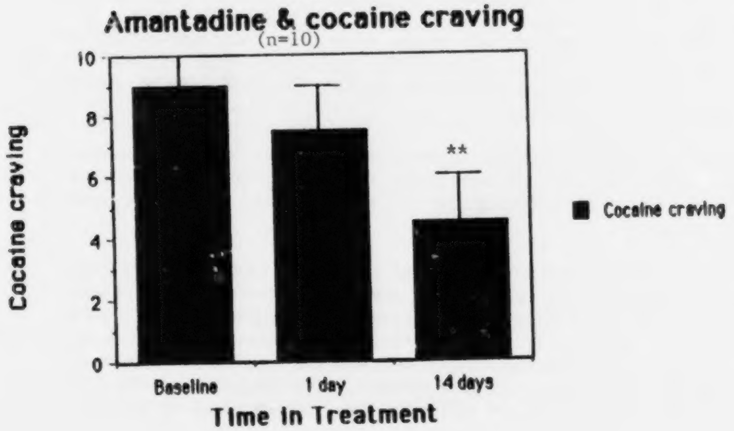
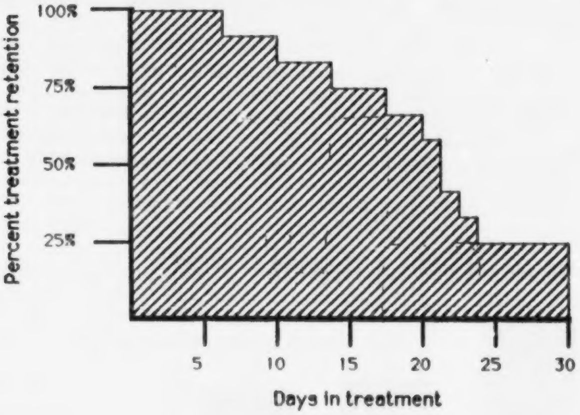


FIGURE 3

Retention on amantadine over 30 days



DISCUSSION

In our study amantadine appeared to be a safe and possibly effective short term treatment for cocaine craving and abuse. While the drug does not appear to have any immediate anti-craving effect, patients who remained on amantadine showed a decreasing trend in their cocaine use and craving. The decrease was for placebo and active treatments of two weeks in other pharmacotherapy trials, and double-blind random assignment trials will be needed to assess amantadine's clinical effect.

Patients in the Tennant and Sagherian study had all used cocaine within 72 hours of entering. We did not adhere to a cut-off, but eight of twelve patients had used cocaine within 72 hours. All of the patients specifically sought treatment with medication or were referred by their clinician for medication trial because they failed to become abstinent on their own or while in standard treatment at the clinic.

While we did not analyze urines for the presence of amantadine, we found that patients accurately reported their cocaine use as confirmed by benzolyecognine and had complied with other aspects of treatment.

Chronic administration of central stimulants causes a protracted anergic withdrawal syndrome in man and a corresponding subsensitivity of the principal dopaminergic center, the nucleus accumbens, in animals. Tricyclic antidepressants have been shown to reverse the supersensitivity in animals and ameliorate abstinence symptoms in chronic cocaine abusers. The tricyclics suffer the disadvantage of 2 - 4 week delay time before onset of action.

While the exact mechanism of action of amantadine is not known, the drug's action may be more rapid than tricyclic antidepressants and may reduce early drop-outs.

Of note is that the majority of our patients remained in treatment up through the third week of study. While this might make amantadine ideal in combination with tricyclics, it raises the question of whether on its own amantadine's effectiveness decreases with time, since side effects cannot explain the delayed drop-out. If amantadine acts presynaptically to release dopamine, then residual dopamine would be required for the drug to act. Long term amantadine administration could therefore exacerbate an already dopamine depleted state created by chronic cocaine use. The role of tyrosine, a norepinephine and dopamine precursor, was not examined in this study, but warrants further consideration.

It is our view that amantadine may be effective in treating the early phase of cocaine withdrawal. Well controlled clinical trials are required before large scale use of the drug can be recommended for the treatment of cocaine abuse.

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These may be obtained from the author on request.

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Behavioral and Neurochemical Effects of Repeated or Continuous Exposure to Cocaine

M. Kleven, W. Woolverton, C. Schuster and L. Seiden

The behavioral effects of cocaine (COC) were studied in rhesus monkeys using a paradigm in which operant behavior was sampled for 1/2 hr every 6 hours and COC was continuously infused via an iv catheter. COC (4-32 mg/kg/day) initially caused reductions in the rate of responding for food and tolerance developed to this effect. When the infusion of COC was terminated following a prolonged period of exposure to the high dose (32 mg/kg/day), there was a marked suppression of operant behavior, lasting as long as 72 hrs, as well as observable changes in behavior (*e.g.* hyporesponsiveness). The long-term neurochemical consequences of exposure were examined in rats with COC administered by continuous iv infusion (100 mg/kg/day for 21 days). There were no significant changes in the concentration of DA or 5-HT 2 weeks after continuous infusion. These results suggest that prolonged exposure to COC does not produce neurotoxicity like that observed with methamphetamine (MA) and amphetamine-like (AMPH-like) compounds.

The behavioral effects of single injections of psychomotor stimulants include locomotor stimulation, reduction in food intake, and stereotypy. These effects are thought to be mediated by enhanced catecholamine neurotransmission in the brain (Lewander 1974; Wise 1984). In contrast, the behavioral and neurochemical effects of repeated or prolonged exposure to COC are not well known. With the recent rapid increase in the use of COC, the effects of long-term exposure to COC have become an important public health issue.

The consequences of repeated administration of a drug may include tolerance or sensitization, dependence, and long-term changes in brain chemistry. Both tolerance (Wood and Emmett-Oglesby 1986; Woolverton *et al.*, 1978a,b) and sensitization (*e.g.*, Post *et al.*, 1976) to the behavioral effects of COC have been reported to develop upon repeated administration. The direction of the change in sensitivity seems to depend upon such variables such as the conditions of drug administration and the behavior being studied (Jones 1984). Whether prolonged exposure to COC results in dependence is more controversial. Although COC has been thought not to produce physical dependence, recent research with COC abusers has revealed a predictable sequence of signs and symptoms (e.g., irritability, depression and anxiety) that occur following prolonged intake of COC (Gawin and Kleber 1986).

The neurochemical consequences of repeated exposure to COC administration have only recently been examined. The similarities in acute neurochemical effects of COC and AMPH-like compounds raise the possibility that repeated exposure to COC might produce long-term neurotoxic changes similar to those produced by AMPH. These effects include, but are not limited to, reductions in the concentrations of dopamine (DA) and serotonin (5-HT) in several brain regions, and are probably the result of destruction of nerve terminals containing these monoamines (Seiden 1985; Ricaurte *et al.*, 1982;1984; Wagner *et al.*, 1983). It has recently been reported that repeated COC administration to rats depleted striatal tyrosine hydroxylase activity 60 days after the last injection (Trulson *et al.*,1986), suggesting that prolonged exposure to COC can produce long-lasting damage to DA-containing neurons. More research is needed before definitive statements can be made concerning the neurotoxicity of COC.

METHODS

Behavioral effects of continuous infusion of COC in rhesus monkeys.

Rhesus monkeys were fitted with stainless steel harnesses and spring arms for restraint and catheter protection. They were housed in experimental chambers containing 2 response levers with stimulus lights and a houselight. Water was continuously available and food was restricted to that obtained during behavioral sessions and supplementary feeding with monkey chow to maintain body weight. The procedure was identical to one used previously to study the effects of continuous infusions of PCP or THC (Beardsley *et al.*, 1986; Slifer *et al.*, 1984). Every 6 hours (10 AM - 4 PM - 10 PM - 4 AM), the house light and lever lights were illuminated for 30 min and responding on the right lever under a fixed-ratio schedule (FR 50 or 100) resulted the delivery of 1 gram banana-flavored food pellets. The FR was held at the maximum level at which typical FR patterns of responding were observed and the monkeys maintained stable body weights with food received during the sessions. Responding on both levers was recorded between periods of food availability.

When behavior was stable, an intravenous catheter was implanted and a continuous i.v. infusion of saline was delivered by a syringe pump. When behavior was again stable, a continuous infusion of COC was begun 2 hours before the 10 AM session on day 1. If COC disrupted behavior, the infusion was continued for a minimum of 10 days, until behavior returned to baseline levels or a new stable level of responding was achieved. If responding remained below baseline levels for 3 consecutive days, the monkeys were given 2 small post-session (10 AM and 4 PM) meals of monkey chow to maintain a consistent level of food-intake. As tolerance developed, doses were increased by doubling to a maximum of 32 mg/kg/day. Saline was then substituted for COC and continued for at least 30 days. The regimen was repeated at least once in each monkey.

Neurochemical effects of continuous administration of COC in rats.

Male Sprague-Dawley rats (Harlan, IN; 250-300g) were housed individually in steel cages with food and water available *ad lib.* Room lights were on a 12 hour dark-light cycle (0700-1900) and temperature was maintained at 24 °C. Rats (n=4) were anesthetized with pentobarbital (50 mg/kg, ip) and the external jugular vein was catheterized using silicone tubing. Catheters were connected to sc implanted alzet® osmotic pumps (2ML4; alza, Palo Alto, CA) containing COC (0.58 mg/ml) resulting in a dose of approximately 100 mg/kg/day. The wounds were closed with Michael clips and the rats were returned to their home cages. A sham group (n=5) was implanted with sealed jugular catheters. After 21 days of continuous COC infusion, pumps were removed under pentobarbital anesthesia. Two weeks later rats were sacrificed by decapitation and the brains were dissected (Heffner *et al.*, 1980). Tissues (30-100 mg) were stored in liquid nitrogen until assayed for DA, DOPAC, HVA, 5-HT, and 5-HIAA by HPLC-EC.

RESULTS

The effects of the various infusion conditions on FR responding for food are summarized as effects on total number of food pellets received over an entire 24 hr period. Although there were occasional irregularities in responding in individual time periods, the effects of COC on responding are accurately reflected by this measure. When saline was infused, 5018 received an average of approximately 150-165 food pellets/day (fig. 1). Monkey 2039 received an average of

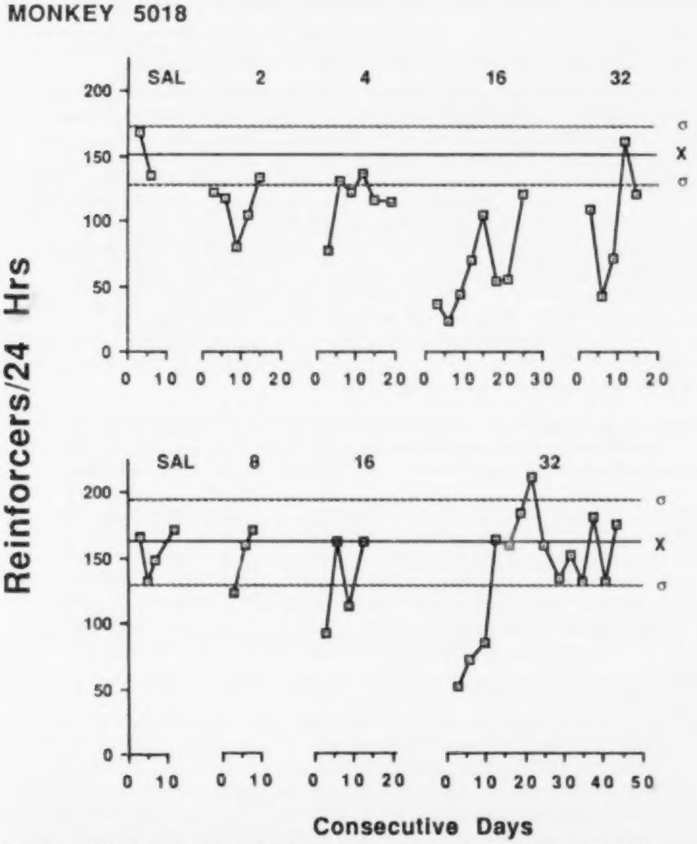

Figure 1. Effect of Continuous Infusion of Cocaine upon FR Operant Behavior in the Rhesus Monkey. Doses of cocaine, shown above the data points, are expressed as mg/kg/day. Each symbol represents the mean number of reinforcers earned during 3 successive days comprised of 4 1/2 hr sessions (10 AM-4PM-10PM-4AM). The solid and dashed horizontal lines represent the mean and 1 SD during saline infusion (N=6-9 days), respectively.

184 pellets/day before the first cycle of COC but his baseline had shifted to an average of 109 pellets/day when the second cycle was begun (fig. 2). Consequently, it was necessary to supplement his food intake to 170 g/day during the second exposure to COC.

Continuous infusion of 2 mg/kg/day COC in monkey 5018 (fig. 1) had little effect on behavior. Higher doses of COC (4-32 mg/kg/day), however, reduced responding initially in both monkeys in a dose-related manner (fig.1 and fig. 2). Observable effects of COC included stimulation of locomotor activity beginning at 4 mg/kg/day. Stereotyped grooming and visual checking were seen at 16 and 32 mg/kg/day. The initial decrease in lever-pressing was followed by a gradual (usually 6-12 days) return to baseline levels of responding, indicative of the development of tolerance. There was also evidence of the development of cumulative tolerance to COC over repeated cycles. For instance, monkey 5018 (fig.

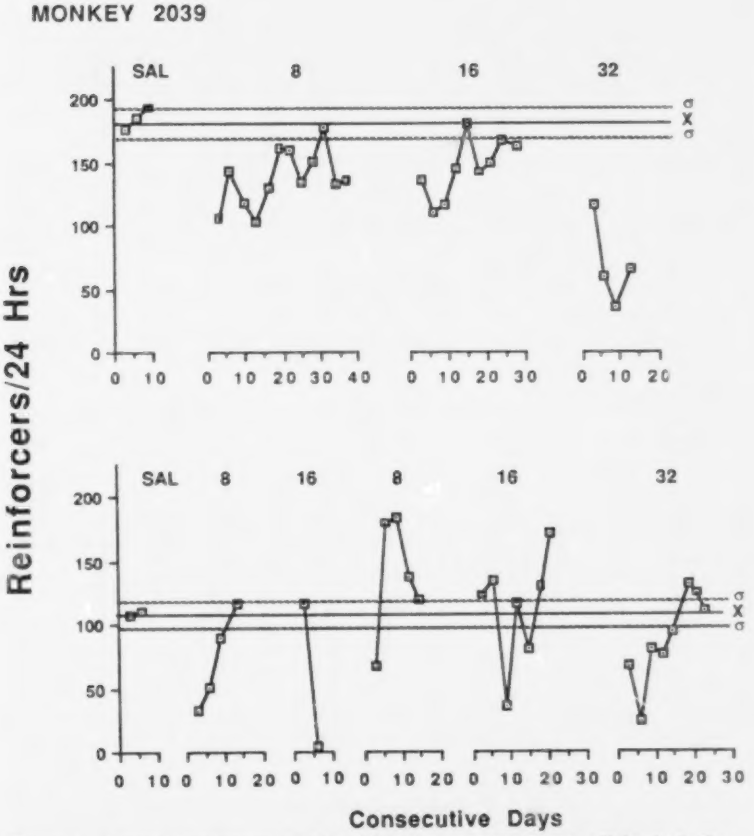

Figure 2. Effect of Continuous Infusion of Cocaine upon FR Operant Behavior in the Rhesus Monkey. See fig. 1 for details.

1, panel A) 16 mg/kg initially suppressed responding for food, while more than 5 months later (panel B) the 16 mg/kg/day dose had minimal effects on behavior. A similar effect was evident in monkey 2039 (fig. 2). Although observable effects of COC were not quantified in this study, their intensity did not appear to change with continuous infusion.

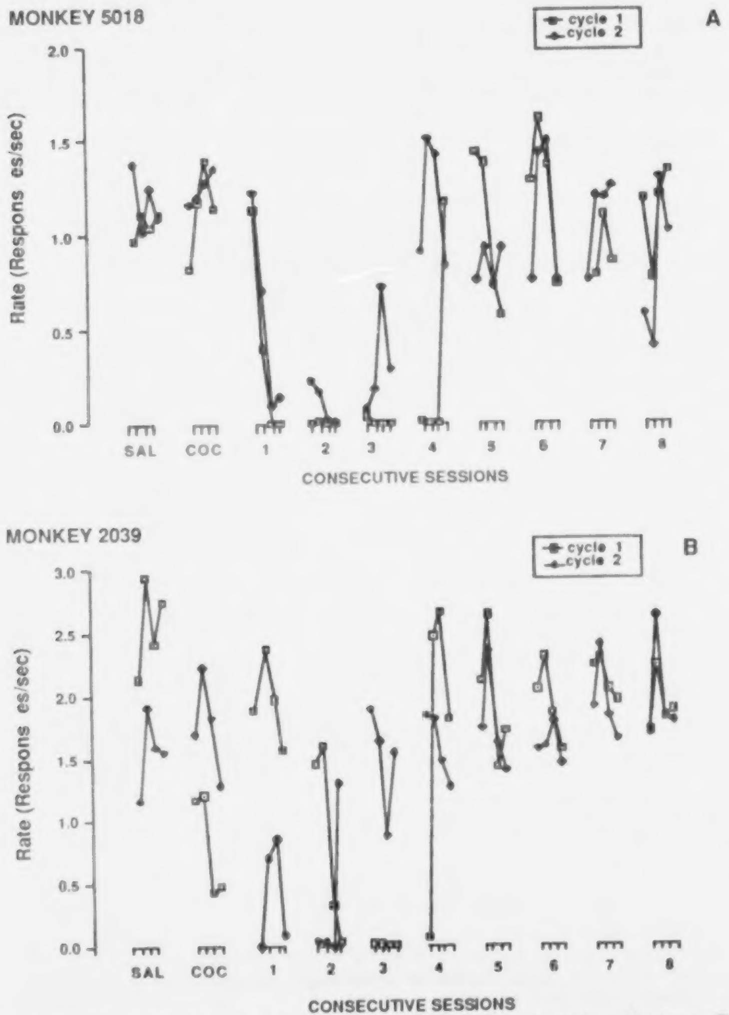

Figure 3. Effect of Withdrawal of Continuous Cocaine Infusion in Rhesus Monkeys. Responding during daily sessions (10AM-4PM-10PM- 4AM) is shown for each day following withdrawal of continuous infusion of cocaine (32 mg/kg/day). The SAL and COC data points represent the means of the last 5 days of saline and cocaine, respectively.

After a period of exposure that varied between 65 and 79 days, 32 mg/kg/day COC was replaced with saline. Decreases in response rate were usually seen beginning in the first day and continued over the next 2-3 days (figs. 1, 2). A detailed analysis of these disruptions is presented in fig. 3. Saline infusions began at 8 A.M. and responding was usually normal for the 10 A.M. session. By the 4 P.M. session on day 1, responding was completely suppressed. In sessions in which responding was not completely suppressed, monkeys usually responded at normal rates for 2-3 food pellets and stopped responding for the remainder of the session. During this period of time monkeys sat in a hunched posture, were hyporesponsive and took offered food. Responding recovered to baseline levels during the third or fourth day following the termination of COC infusion.

Table 1 shows the levels of monoamines and metabolites in rats treated with continuous COC for 21 days at a dose of 100 mg/kg/day. No significant depletions of 5-HT, DA or DOPAC were observed in any of the regions after continuous administration. Continuous infusion of a higher dose of COC (200 mg/kg/day) was lethal within 4 days and could not be tested further.

TABLE 1. Effect of Continuous Infusion of Cocaine (100 mg/kg/day) for 21 Days on Regional Levels of Monoamines and Metabolites.

	Cortex	Hippocampus	Hypothalamus	Caudate
5-HT				
Sham	0.32±0.01[a]	0.42±0.03	0.89±0.04	0.57±0.11
COC	0.43±0.05	0.48±0.04	1.04±0.06	0.60±0.10
5-HIAA				
Sham	0.22±0.01	0.35±0.01	0.56±0.02	0.59±0.05
COC	0.40±0.13	0.37±0.04	0.68±0.03	0.60±0.07
DA				
Sham	ND[b]	ND	0.33±0.04	13.57±0.48
COC	ND	ND	0.49±0.13	15.00±0.66
DOPAC				
Sham	ND	ND	ND	1.73±0.14
COC	ND	ND	ND	1.79±0.22

[a] mean±SE, μg/g wet weight; [b] ND-not determined

DISCUSSION

Continuous infusion of COC had several behavioral effects in rhesus monkeys. The rate of responding under the FR schedule was reduced by COC in a dose-related manner, as has been reported previously with single injections (MacPhail and Seiden 1975; Woolverton *et al.*, 1978a). In addition, locomotor stimulation and, at the high doses (16 and 32 mg/kg/day), stereotyped behaviors were observed. As the COC infusion was continued, there was no apparent change in sensitivity to the directly observable effects of COC. However, tolerance developed to the effect of COC on FR responding as evidenced by a return of response rate to baseline levels generally over 5 to 15 days. As COC dose was increased, tolerance developed repeatedly until the monkeys were responding at baseline rates with a continuous infusion of 32 mg/kg/day. Partial tolerance to COC has been reported previously using single injections but required 45-60 days to develop (Woolverton *et al.*,1978a,b). Tolerance has also been reported to develop to the discriminative stimulus effects of COC when the drug was administered twice daily for 7 days (Wood and Emmet-Oglesby 1986). Thus, it seems that more frequent exposure to COC leads to more rapid and complete tolerance development. The findings of the present experiment extend the conditions under which tolerance develops to COC.

An additional factor apparent in the present study is the development of what might be termed a "cumulative" tolerance to COC. When the continuous infusion regimen was repeated, tolerance developed more rapidly than it had previously. Evidence for this fact can be seen particularly at the dose of 16 mg/kg/day in figs. 1 and 2. In addition, in pilot studies designed to establish the parameters used in the present experiment, doses of COC as low as 8 mg/kg/day completely suppressed behavior (data not shown) but had little or no effect later in the same animals. Such an effect, if born out in further studies, has implications for the dose escalation that may be necessary for COC dependence to develop.

When continuous infusions of 32 mg/kg COC were terminated, a consistent set of signs and symptoms were observed that suggested a withdrawal syndrome resulting from COC dependence. Operant behavior was disrupted to the point of complete suppression. The monkeys became hyporesponsive, sat in a hunched posture and locomotor activity was much reduced. The time course was similar for all of these effects. They began to appear within 6 hours after termination of the COC infusion, and recovery began to occur approximately 48 -72 hours after termination of the infusion. This time course is similar to that reported by Gawin and Kleber (1986) or the "crash" period in human COC abusers. Thus, it is possible that this may represent a useful paradigm for the laboratory investigation of the effects of withdrawal from a binge of COC self-administration. It is interesting to note in this context that rhesus monkeys given a choice between an injection of COC (0.3 mg/kg/inj) or food pellets every 15 minutes 24 hours/day continuously for 8 days self-administered up to approximately 28 mg/kg/day of COC (Aigner and Balster 1978). Thus, the dose of 32 mg/kg/day used in the present study is clearly in the range of doses self-administered by monkeys given the option to respond for an alternative reinforcer. Research is underway to further assess the utility of this animal model for studying COC dependence.

It is important to establish the conditions under which COC dependence develops. The present results using a continuous infusion paradigm suggest that both dose and duration of exposure are of critical importance. Such a conclusion would be consistent with what is known about dependence upon other psychoactive compounds. In pilot studies, signs or symptoms of withdrawal from COC were not seen with infusion doses of less than 32 mg/kg/day. The most intense behavioral disruptions (cycle 1, monkey 5018) followed the most prolonged period of continuous COC infusion and exposure to the high dose, thus demonstrating withdrawal from COC. It seems to be necessary to gradually escalate to this high dose of COC by developing tolerance to the low doses first. In pilot studies, monkeys that were infused directly with 16 mg/kg/day COC without first becoming tolerant to a lower dose failed to become tolerant to 16 mg/kg/day. In this context, the "cumulative" tolerance alluded to previously becomes an important phenomenon. If it becomes increasingly easy to achieve the prolonged exposure to a high dose of COC, the probability of dependence developing in a relatively brief period of time becomes more likely. Further research is needed to establish the generality of this finding.

Continuous infusion of a high dose of COC (100 mg/kg/day) for 21 days failed to alter DA and 5-HT. Roy *et al.*. (1978) also found that acute doses of COC failed to alter DA content in the caudate nucleus after repeated injections of COC (30 mg/kg/day for 30 days). On the other hand, Taylor and Ho (1977) reported that repeated administration of COC (10 mg/kg/day for 7 days) caused a decrease in both 5-HT levels and tryptophan hydroxylase activity in the caudate and an increase in striatal DA and NE metabolism which lasted for 24 hrs. Since previous investigators have examined neurochemical effects within 24 hrs of the last injection of COC, it is impossible to determine whether these effects were long-lasting. More recently, Trulson *et al.* (1986) reported that tyrosine hydroxylase immunoreactivity in the straitum was reduced by repeated administration of COC for up to 60 days after the last injection. Such an effect on tyrosine hydroxylase is consistent with toxicity to catecholamine-containing neurons. The reasons for the apparent discrepancy with the present results is unclear. It is possible, however, that tyrosine hydroxylase activity was reduced after the chronic regimen but that steady-state levels of neurotransmitter were unchanged.

Our results with COC are in contrast to what has been found with AMPH-like psychomotor stimulants. Both MA and d-AMPH deplete monoamines in at least one of the brain regions examined here. Although the precise reason for this difference between COC and AMPH is unclear, there are several pharmacological differences between the two compounds that may play a role. For instance, it has been postulated that the production of endogenous neurotoxins plays a role in the neurotoxicity produced by MA (Seiden and Vosmer 1984). It is possible that such a neurotoxin is produced but that COC limits it's own toxicity by blocking the uptake of the neurotoxin into the relevant neurons. Additionally, COC does not cause release of DA and may, therefore, be self-limiting with regard to elevating synaptic concentrations of neurotransmitter, thereby reducing production of neurotoxin. In this context, the lack of MAO inhibition by COC as compared to AMPH may also limit the availability of endogenously produced neurotoxin. Finally, it has been suggested that COC, like methylphenidate, acts via a different neurotransmitter pool than AMPH, a pool that does not depend upon recent synthesis (Scheel-Kruger 1977). The observations that α-methyltyrosine, which inhibits catecholamine synthesis, blocks the neurotoxicity of AMPH (Wagner, *et al.*, 1983) and that methylphenidate does not cause AMPH-like neurotoxicity (Wagner *et al.*, 1981) are consistent with this hypothesis.

In summary, continuous exposure to COC results in tolerance to its effects on FR responding for food. When continuous infusions of COC were terminated after a period of exposure during which the dose was escalated to 32 mg/kg/day, a reliable sequence of behavioral changes suggestive of a withdrawal syndrome were evident. COC is thus similar to other psychoactive drugs in its ability to produce what has been termed "behavioral dependence" (Schuster 1968). In addition, high doses of COC did not produce the damage to monoamine containing neurons in the brains of rats that has been seen with AMPH-like compounds. It should be emphasized that the lack of AMPH-like neurotoxicity does not preclude the existence of some other form of toxicity to the brain.

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Neurochemical Basis for Cocaine and Methamphetamine Interactions

G. Hanson, L. Matsuda and J. Gibb

ABSTRACT

We have evaluated the responses of rat monoaminergic pathways to combinations of cocaine and methamphetamine (METH) administrations and found that these two commonly abused stimulants significantly interact. A 7-day pretreatment with daily doses of cocaine enhanced the neurochemical changes induced by multiple doses of METH in the dopaminergic and serotonergic systems of the basal ganglia and frontal cortex. When cocaine was administered concurrently, it attenuated or blocked the serotonergic changes caused by multiple doses of METH, but it did not interfere with the METH-mediated changes in the dopaminergic system.

INTRODUCTION

The commonly abused stimulants, cocaine and amphetamines, are frequently self-administered in toxic doses, resulting in profound CNS effects. Many of the neurochemical changes which result from the separate administration of high doses of each of these potent drugs have been previously described. It is well documented that a significant portion of the stimulant abuser population consists of polydrug users who administer both drugs either alternately or even concurrently (Cohen, 1975; Siegel, 1984); however, little information is available concerning the interactions of these agents. Previous reports that administrations of both stimulants influence the neurochemistry of similar monoaminergic systems, associated with structures of the basal ganglia and mesocortical areas (Pradhan et al., 1978; Pradhan, 1983; Goeders and Smith, 1983; Morgan and Gibb, 1980; Schmidt et al., 1985), suggest that significant interactions are likely to occur and could influence toxic and pharmacological responses to these agents.

We have evaluated the responses of dopaminergic and serotonergic pathways, associated with either the basal ganglia or mesocortical structures, to combinations of cocaine and methamphetamine (METH) and found that the METH-induced changes in related neurochemical parameters are significantly altered by cocaine treatments.

MATERIALS AND METHODS

Drug treatments: Male Sprague-Dawley rats (200-250 gm) were housed 5 per cage and maintained at 26° C with a 12-hr alternating light-dark cycle. Both cocaine and METH were dissolved in 0.9% saline and administered intraperitoneally and subcutaneously, respectively. Doses are expressed in terms of the free base. Cocaine pretreatments consisted of a daily dose (20 mg/kg) for 7 days administered prior to 3 doses (15 mg/kg/dose; 6 hr-intervals) of METH. Animals were sacrificed 18 hr following the final drug administration. Concurrent drug treatments were administered by injecting 5 doses (6-hr intervals) of cocaine (20 mg/kg) and METH (15 mg/kg) at approximately the same time and sacrificing the animals 18 hr after treatment.

Assays: Following decapitation, the brains were immediately removed and placed on ice. The neostriata and frontal cortical regions were dissected bilaterally and stored at -70° C until assayed. One of the paired striata or cortical regions from each rat was assayed for tryptophan hydroxylase (TPH) and tyrosine hydroxylase (TH; striata only) enzyme activities while the other was used in the quantitation of the monoamines and their metabolites.

All steps in the preparation of enzymes were performed at 0-5° C. Tissues were weighed and homogenized (1:3) in 50 mM HEPES buffer, pH 7.4, containing 0.2% Triton X-100 and 5 mM dithiothreitol. Homogenates were centrifuged at 27,000 x g for 15 minutes. Duplicate 7.5-µl aliquots of the supernatant fraction from each sample were analyzed for TH activity by a tritium release assay (Nagatsu et al., 1964) utilizing ^{3}H-tyrosine as substrate. Similar aliquots were assayed for TPH activity using a modified $^{14}CO_2$-trapping procedure (Ichiyama et al., 1970); details of the assay are described by Hotchkiss et al. (1979).

Tissue concentrations of dopamine (DA) and its primary metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and 5-hydroxytryptamine (5HT) and its major metabolite, 5-hydroxyindoleacetic acid (5HIAA), were measured by high-performance liquid chromatography (HPLC) with electrochemical detection. Tissues were weighed and homogenized in 0.3-0.5 ml mobile phase buffer containing 0.15 M monochloroacetic acid, 2.0 mM disodium EDTA and 25 mg/l 1-octane sulfonic acid in 12.5% methanol at pH 2.9. After centrifugation at 40,000 x g for 15 min the supernatant fractions were filtered through a 0.2-µm Microfilter system (Bioanalytic Systems, Inc., West Lafayette, IN). Fifty-µl volumes were injected by a WISP automatic sample processor (Millipore Co., Millford, MA) onto a 3-µm reverse phase Microsorb C_{18} column (Rainin Instrument Co., Woburn, MA). Samples were run on a LC-154 liquid chromatograph equipped with a model LC-4B amperometric detector (Bioanalytical Systems, Inc.). The detector potential was set at +0.73 V. Monoamines and their metabolites were quantitated by measurement of peak heights and comparison with those of standards of known concentration prepared in mobile phase buffer.

RESULTS

Pretreatment with single daily doses of cocaine for 7 days had no significant neurochemical effects alone, but did substantially increase neurochemical responses by monoaminergic systems to multiple doses of METH (figure 1). Specifically, cocaine pretreatment enhanced the METH-induced decreases in striatal TPH activity (figure 1A), striatal and cortical concentrations of 5HT and 5HIAA (figures 1 B and C) and striatal concentrations of DA and DOPAC (figure 1D).

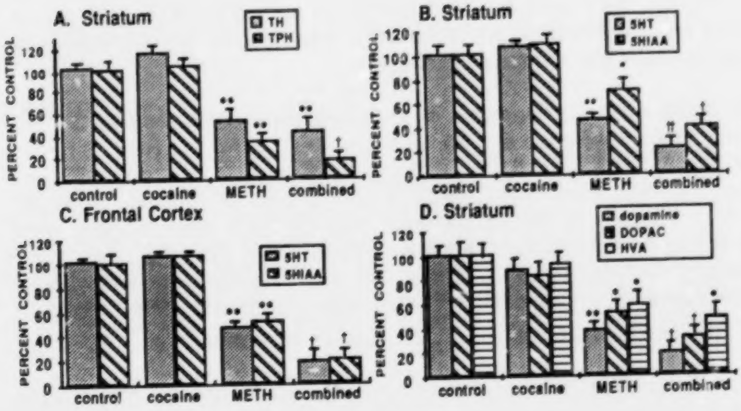

FIGURE 1. Effects of cocaine pretreatment on METH-induced changes in (A) striatal TH and TPH activities, (B and C) concentrations of 5HT and 5HIAA in striatum and frontal cortex, respectively, and (D) striatal concentrations of DA, DOPAC and HVA. Each value represents 4-10 animals. * $P < 0.02$, ** $P < 0.01$ compared to respective controls; † P <

0.05, †† $P < 0.02$ compared to corresponding METH-treated groups. Control values for striatal tissue were: TH=3080, TPH=33.9 nmol/g/hr; 5HT=0.441, 5HIAA=0.349, DA=10.8, DOPAC=1.55, HVA=0.82 µg/g tissue. Controls for cortical tissue were: 5HT=0.353, 5HIAA=0.212 µg/g tissue.

Interactions between cocaine and METH also were observed when multiple doses of these agents were administered concurrently (figure 2). Multiple doses of cocaine alone significantly decreased the activities of striatal TH and TPH (figure 2A). The presence of cocaine substantially attenuated or blocked the effects of METH on striatal and cortical TPH activities (figures 2A and B), as well as striatal and cortical concentrations of 5HT and 5HIAA (figures 2C and D). In contrast, concurrent cocaine treatment did not alter the effects of METH on striatal TH activity (figure 2A) or striatal concentrations of DA, DOPAC or HVA.

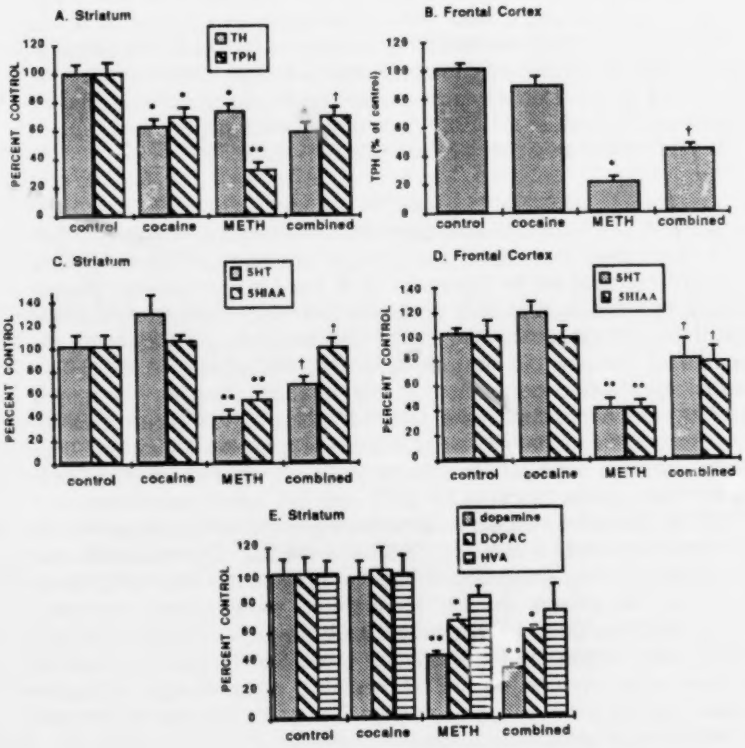

FIGURE 2. Effects of concurrently administered cocaine on METH-induced changes in (A) striatal TH and TPH activities, (B) cortical TPH activity, (C and D) concentrations of 5HT and 5HIAA in striatum and frontal cortex, respectively, and (E) striatal concentrations of DA, DOPAC and HVA. Each value represents 4-6 animals. * $P < 0.02$, ** $P < 0.005$ compared to respective controls. † $P < 0.01$ compared to corresponding METH-treated groups.

DISCUSSION

These findings demonstrate that there exists substantial potential for interactions between cocaine and METH and the nature of such interactions vary according to dosing protocols. Chronic administration of cocaine has been shown to cause sensitization to itself in rats (Post and Contel, 1983), dogs, monkeys (Tatum and Seevers, 1922) and humans (Jones, 1984). Results from the present study suggest that cross-sensitization between cocaine and METH also occurs. Thus, cocaine pretreatment for a week enhanced the response of monoaminergic systems associated with the striatum and frontal cortex to METH treatment. The mechanism for this cocaine-induced sensitization is not presently apparent. However, our previous observations that many of the METH-related effects are due to the actions of this drug on DA-release (Schmidt et al., 1985) would suggest a dopaminergic basis for the cocaine-induced sensitization to METH action.

The changes observed with METH effects, when cocaine was administered concurrently, were particularly interesting. Cocaine significantly attenuated the striatal and cortical serotonergic effects caused by mulitple doses of METH (figure 2A, B, C and D): a similar, but greater blockade by cocaine also was observed following a single concurrent administration of these two stimulants (data not shown). In contrast, the presence of cocaine did not interfere with the effects of METH on dopaminergic-related parameters in the striatum, such as TH activity (figure 2A) and concentrations of DA, DOPAC and HVA (figure 2E). In fact, subsequent experiments suggest that the presence of cocaine may actually enhance such METH actions (data not shown). It has been reported that uptake blockers for 5HT and DA, when coadministered with METH, block the respective dopaminergic and serotonergic effects of this agent (Schmidt and Gibb, 1985a and 1985b). Consequently, the 5HT-uptake blocking properties of cocaine (Taylor and Ho, 1978) likely account for its effects on METH-mediated serotonergic changes. Cocaine also has been characterized as a potent DA-uptake blocker (Hadfield and Nugen, 1982; Groppetti et al., 1973), but the lack of interference by cocaine on METH-mediated dopaminergic changes suggests that the effect of cocaine on the DA uptake complex is different from that of other dopaminergic uptake blockers.

In summary, significant interactions occur between cocaine and METH ranging from an apparent sensitization to METH effects by cocaine pretreatment to a blockade of METH-induced 5HT changes caused by concomitant cocaine administration. These interactions could have clinical significance to polydrug users who abuse both drugs as well as help to elucidate the mechanisms of action of these compounds.

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Chronic Cocaine Modifies Brain Benzodiazepine Receptor Densities

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INTRODUCTION

Investigations of both the direct and indirect neurobiological effects of acute and chronic cocaine administration may lead to a greater understanding of compulsive drug use and associated behavioral pathologies. Since cocaine increases the availability of catecholamines in the synapse by blocking reuptake into presynaptic neurons, most attention has focused on its dopamine-mediated reinforcing and stimulant properties (see Woods et al., 1987). For example, the administration of alpha-flupenthixol (a dopamine receptor antagonist) attenuates intravenous cocaine self-administration (Ettenberg et al., 1982), and depletion of dopamine in the nucleus accumbens by 6-hydroxydopamine (6-OHDA) decreases cocaine self-administration (Roberts et al., 1980) as well as cocaine-induced hyperactivity (Kelly and Iversen, 1976).

Chronic treatment with psychomotor stimulants can result in long lasting changes in catecholaminergic systems although there is some confusion as to the exact nature of these effects (Post et al., 1987). Thus, daily injections of amphetamine have been reported to either increase (Akiyama et al., 1982) or decrease (Hitzemann et al., 1980) dopaminergic receptor binding in mesolimbic areas. Increases in ^{3}H-spiroperidol binding have also been reported in frontal cortical areas (Akiyama et al., 1982) with decreases found in the striatum following amphetamine treatment (Howlett and Nahorski, 1979; Akiyama et al., 1982). Cocaine increases [^{3}H]-spiroperidol binding in the striatum (Taylor et al. 1979; Memo et al., 1982) but decreases the binding of sulpiride, a relatively specific ligand for the D_2 receptor (Goeders and Kuhar, 1987). Sulpiride binding is increased in the nucleus accumbens (Goeders and Kuhar, 1987).

Since changes in brain catecholamines may, in turn, result in additional central nervous system alterations, it may be possible for chronic cocaine administration to induce neurobiological modifications indirectly through its effects on catecholamine systems. There is evidence that catecholaminergic systems mediate changes in benzodiazepine (BZD) receptor densities. Depletion of brain dopamine by intraventricular 6-OHDA decreases BZD receptor densities in the cerebral cortex (Sabato et al, 1981) and cerebellum (Noble et al., 1981). Systemic pretreatment with buspirone, a non-benzodiazepine anxiolytic which blocks dopamine receptors (McMillen et al., 1983), increases [^{3}H]flunitrazepam and [^{3}H]Ro 15-1788 labeling of BZD receptors in the cerebral cortex, cerebellum and hippocampus in vivo (Oakley and Jones, 1983; Goeders et al., 1986). Furthermore, chronic cocaine administration has been reported to affect ɣ-aminobutyric acid (GABA) receptor binding in the rat striatum (Gale et al., 1981). Therefore, cocaine-induced alterations in catecholaminergic and non-catecholaminergic neurotransmission may result in changes in BZD receptor populations.

A complex relationship between stimulants and BZD receptor ligands has been implicated in behavioral studies. Systemic chlordiazepoxide injections significantly decrease the frequency of cocaine self-infusions by rats (Goeders and Dworkin, 1987) in a fashion similar to that seen with dopaminergic antagonists (Ettenberg et al., 1982). These latter results were interpreted as a modification in the reinforcing efficacy of cocaine. Therefore, BZD receptors may be involved in the neurobiological events mediating cocaine reinforcement. In addition, co-injections of cocaine and chlordiazepoxide augment locomotor activity above that of the two drugs alone (D'Mello and Stolerman, 1977). Chlordiazepoxide pretreatment also enhances the stimulatory effect of chlordiazepoxide and amphetamine combinations (Sansone et al., 1986). Acute administration of amphetamine has been demonstrated to potentiate the anticonflict properties of chlordiazepoxide (Lerner et al., 1986) as does chronic administration of chlordiazepoxide-amphetamine mixtures (Ford et al., 1979). Cocaine generalizes to the anxiogenic agent, pentylenetetrazol, and this generalization is antagonized by diazepam (Shearman and Lal, 1979). These data suggest that cocaine may have anxiogenic properties that could be influenced by drugs specific for the BZD receptor. Indeed, clinical reports have described anxiety reactions that are treatable with diazepam following high doses or chronic cocaine (Gay, 1982).

Therefore, both neurobiological and behavioral data suggest a complex interaction between stimulants and BZD's and the neural substrates at which each act. The present study investigated the effects of cocaine administration upon BZD receptors since data of potential clinical relevance in the treatment of compulsive cocaine use and associated behavioral pathologies may be revealed.

METHODS AND MATERIALS

Twelve adult male rats (90-120 days old) originally derived from the Fischer 344 strain were injected with cocaine (20.0 mg/kg, i.p.) or an equal volume of physiological saline (1.0 ml/kg, i.p.) for fifteen days. On the fifteenth day, the rats were sacrificed by decapitation twenty minutes post-injection, and the brains were removed and dissected over ice into cerebellum, cerebral cortex, diencephalon, brain stem, hippocampus and striatum. The dissected brain regions were frozen on dry-ice and stored at -70°C until assay. Membranes were prepared by tissue homogenization (Polytron, at setting P-10 for 15 seconds) and centrifugation (15000 rpm for 10 minutes) in 15 ml of ice-cold 50 mM Tris-HCl (pH 7.7) two times, with the supernatant discarded. The final membrane pellet was diluted to 1.7 mg/ml (approx. 150 ug protein/ml). The binding assay consisted of incubating triplicate samples of tissue (1.0 ml) at 4°C for 60 minutes with [^{3}H]Ro 15-1788 (10^{-9}M) and various concentrations of "cold" Ro 15-1788 (5×10^{-10}M to 10^{-6}M) or buffer. The reactions were terminated by filtration. Binding was determined by liquid scintillation spectrophotometry, and specific binding was calculated as the difference between total and non-specific binding. Protein content of the tissue samples was assessed using the Lowry method (Lowry *et al.*, 1951), and Scatchard and Hill plots were estimated by computer-aided regression analysis (McPherson, 1983) resulting in the binding affinities (Kd) and densities (Bmax) for the tissue for each animal. Independent t-tests were used to compare differences in Bmax and Kd values obtained from the treatment groups.

RESULTS

Daily injections of cocaine resulted in increases in activity and stereotypy over the fifteen day period. Figure 1 shows the changes in behavior rated for one-hour post-injection for rats in a preliminary study, according to a modification of the index provided by Ellinwood and Balster (1974). Over the first few days,

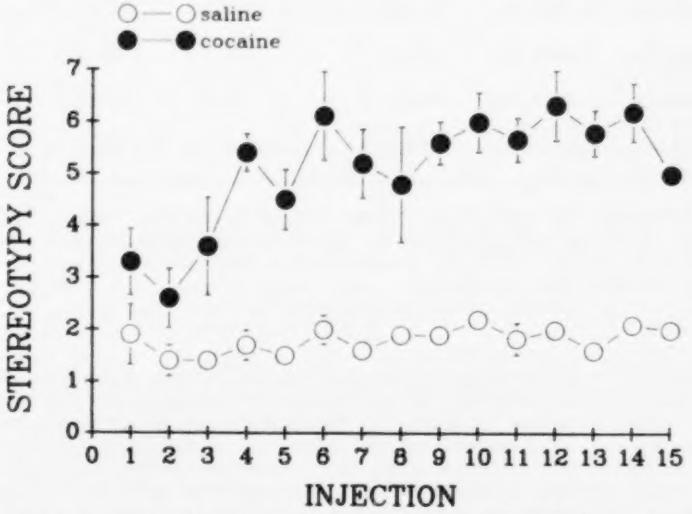

FIGURE 1. Behavioral effects of cocaine (20 mg/kg) over a fifteen day treatment period. Values represent mean (±S.E.M.).

increases in locomotor activity and rearing were recorded, but later in the treatment period, head weaving was the predominant activity observed. Although a detailed record of behavior was not made in the present study, similar changes over the fifteen day treatment were noted.

Chronic cocaine injections resulted in significant increases in BZD receptors labeled with [^{3}H]Ro 15-1788 in the striatum and cerebellum, significant decreases in the frontal cortex and no significant effects in the other brain regions (Table 1). These increases in binding were not due to changed affinity of the receptor for the ligand since the Kd in each area was not significantly different (Table 1).

TABLE 1. Specific [^{3}H]Ro 15-1788 binding to membrane homogenates of various brain regions of saline- and cocaine- treated rats

	SALINE		COCAINE	
Brain Region	Kd (10^{-10}M)	Bmax (fmol/mg protein)	Kd (10^{-10}M)	Bmax (fmol/mg protein)
Striatum	4.33±0.33	397±18.5	4.87±0.27	492±38.7*
Cerebellum	2.44±0.46	471±21.4	3.69±1.33	544±17.2*
Cortex	5.22±0.30	876±26.6	5.22±0.40	800±15.0*
Diencephalon	5.30±0.64	534±49.3	5.74±0.69	493±44.6
Hippocampus	4.28±0.48	796±32.3	3.77±0.61	792±51.4
Brain Stem	4.87±0.48	167±16.7	4.97±0.72	164± 3.0

Mean (±S.E.M.) Kd and Bmax values for each brain region from animals injected with saline or cocaine (20 mg/kg, i.p.) for fifteen days (N=6). *$p<0.05$, independent t-tests.

DISCUSSION

The changes in behavior over the testing period were similar to those described by Post and Contel (1983) and Ellinwood and Balster (1974) after repeated cocaine or amphetamine administration and reflect a behavioral sensitization which may be mediated by mesolimbic dopaminergic pathways (Fog, 1972; Randrup and Munkvad, 1970; Kelly, 1977). These behavioral data indicate that cocaine-induced changes in BZD receptors parallel modifications in dopamine receptors after similar stimulant administration (Akiyama et al., 1982; Goeders and Kuhar, 1987).

The changes in BZD receptors labelled with $[^3H]$Ro 15-1788 were not attributable to changes in the binding affinity of the receptors since there were no significant differences in Kd values for tissue derived from saline- and cocaine-treated animals. Therefore, the differences in Bmax likely result from changes in the BZD receptor densities in these brain regions. These preliminary data therefore support the hypothesis that chronic cocaine administration can modify BZD receptor populations. The mechanism mediating these changes is currently under investigation in this laboratory.

While these data suggest that chronic cocaine administration may affect BZD receptors, the significance of these changes is unclear. Most interest in BZD receptors and endogenous ligands has centered on physiological and behavioral responses to procedures predictive of "stress" and "anxiety". BZD agonists increase punished responding (Geller and Seifter, 1962), conditioned emotional responses (Millensen and Leslie, 1974), punished drinking (Vogel *et al.*, 1971) and rodent social interactions suppressed by environmental novelty (File and Hyde, 1978). Forced swimming (Medina *et al.*, 1986) and conditioned emotional response (Lane *et al.*, 1982) result in significant changes in BZD receptor densities in the cerebellar cortex and hippocampus. Whether or not cocaine can affect emotionality or the behavioral responses to stressful stimuli, these changes in BZD receptors open a new line of investigation into the underlying neurobiology of the behavioral effects of cocaine. Certainly, the changes seen in cocaine self-administration after chlordiazepoxide treatment (Goeders and Dworkin, 1987, and unpublished observations) suggest an important link between the reinforcing efficacy of cocaine and BZD mechanisms. Although further investigation is needed, these data suggest a potential role for BZD therapy in compulsive cocaine use and associated behavioral pathologies.

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Flurothyl Seizure Thresholds Associated with Acute and Chronic Chlordiazepoxide Dependence and Withdrawal

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Tolerance to the anticonvulsant actions of the benzodiazepines is a well recognized clinical problem (cf. Frey et al., 1986). When it develops clinically, the dose may be increased to restore response. However, such a strategy might further stimulate tolerance and also promote physical dependence which is dose dependent (Lukas and Griffiths, 1984). Indeed, functional anticonvulsant tolerance and physical dependence might share a common adaptational neuronal mechanism. Although tolerance might not always be associated with physical dependence, physical dependence is invariably associated with tolerance for most dependence producing drugs. Benzodiazepine anticonvulsant tolerance and physical dependence have not been studied together.

Chemoconvulsant testing provides a single dimension of CNS exictability to quantitate initial anticonvulsant efficacy, chronic tolerance and proconvulsant changes during withdrawal. Flurothyl, a rapidly reversible inhalation convulsant, is particularly suited for this purpose because it produces sub-lethal seizures allowing repeated testing in the same animal.

The objectives of this study are: (1) to evaluate functional anticonvulsant tolerance over a range of chronic benzodiazepine dose levels known to produce progressive dependence based on spontaneous withdrawal signs; (2) to evaluate seizure thresholds when spontaneous withdrawal is maximal; (3) to probe for an association between the magnitude of functional tolerance and dependence based on the flurothyl threshold criterion and (4) to determine if seizure thresholds are reduced for acute (single-dose) dependence precipiated by Ro 15-1788 (Boisse et. al., 1986, a,b).

METHODS

Male Sprague-Dawley rats (Charles River, Wilmington, MA), 325-425g were pair housed under standard conditions for this study and randomly assigned to treatments.

Chronic Chlordiazepoxide Dose-Response Studies: Rats were treated with chlordiazepoxide HCL intragastrically twice daily at 8:00 a.m. and 6:00 p.m. at a dose level of 2.5, 5, 20, 75 or 150 mg/kg. Animals received chronically the same dose they received acutely. Concentrations of drug were adjusted to a constant dosage volume of 5 ml/kg. Concurrent controls received water.

Immediately before and four hours after the first (acute) or last (chronic) dose, rats were tested for CNS depression according to the TDP score criterion described below and 100 ul of whole blood was collected and frozen until analyzed by high performance liquid chromatography (HPLC). Thirty minutes later at t=4.5 hours, rats were tested for flurothyl seizure threshold as described below. Following the last chronic chlordiazepoxide dose, rats were tested daily for overt signs of withdrawal until tested for flurothyl seizure threshold when spontaneous withdrawal was near maximal (based on historical time-course data).

Acute Chlordiazepoxide Dependence Study: Acute chlordiazepoxide dependence was induced by a single intragastric dose of 450 mg/kg p.o. Withdrawal was precipitated 76 hours later by Ro 15-1788 (25 mg/kg, i.p.). Flurothyl seizure threshold testing was begun 2 minutes after Ro 15-1788 injection.

Pretreatment controls received water; antagonist controls received the acacia vehicle.

Flurothyl Seizure Threshold Testing: Seizure thresholds were determined by administering flurothyl (bis-(2,2,2,trifluroethyl)ether) utilizing an inhalation technique similar to Adler (1975). Briefly, the rat is placed in a 4L closed chamber equipped with a wire mesh basket on which a 2" x 2" gauze pad is placed. Constant rate infusion (0.11ml/min) of a 10% or 25% solution of flurothyl in 95% ethanol (v/v) was begun. The animal is continuously observed and the time to the first myoclonic jerk and clonic seizure (loss of posture) recorded. A minimum of 5 min. was allowed between animals so that the chamber air could become free of flurothyl vapor and to change the gauze pad.

Based on the concentration of the HFE solution, specific gravity of flurothyl (HFE) and the infusion rate, delivered dose was calculated. The response threshold (MJ or CL) is dose of flurothyl (mg) necessary to overcome the anticonvulsant action of chlordiazepoxide. To allow for comparison between acute and chronic for the dose-response study, net HFE scores were determined by subtracting the control group mean from individual test animal scores for the appropriate response and timepoint. All seizure testing was done between 10 a.m. and 2 p.m. to eliminate any diurnal influences that affect chemoconvulsant susceptibility.

TDP Score Evaluation: CNS depression was evaluated based on five different ladder and open-field tests to detect motor impairment.

The sum tally of all grades of impairment for the 5 tests comprise the total depression point (TDP) score. The maximum TDP is 15. Detailed descriptions of this method have been published (Boisse et al, 1986a).

WD Score Evaluation: Whole animal observations of withdrawal signs were made by two concurrent experienced raters who were blind to the chronic treatment. Twenty different motor, autonomic and behavioral signs were monitored by operationally defined criteria that have been described (Ryan and Boisse, 1983; Boisse et al, 1986a). The WD score is the sum of all grades for all signs recorded.

Quantitation of Benzodiazepine Blood Levels: Blood levels of drug were monitored at the time of peak effect for the first and last dose of the chronic dose-response study. Drugs were extracted from blood using the Bond-Elut system. Chlordiazepoxide and its active metabolites -- nor-chlordiazepoxide and demoxepam -- were quantified based on an HPLC method developed in this lab (Boisse and Rivkin, 1981).

RESULTS

Acute versus Chronic Dose-Response Study

(a) Dose-Defined Tolerance: All acute doses of chlordiazepoxide produced a significant elevation of HFE thresholds compared to controls. The CL criterion curve was just above the parallel to the MJ criterion curve for both acute and chronic doses. An analysis of HFE thresholds in acute and chronic water controls revealed no significant difference for either MJ or CL criteria. Therefore, the evaluation of chronic anticonvulsant tolerance was not confounded by sensitization or altered responsivity to HFE.

Compared to the acute, the chronic dose-response curve was just below the acute curve for both ML and CL with the exception of one data point -- the 75 mg/kg dose and CL criterion. Dose-defined anticonvulsant tolerance was statistically significant for MJ criterion at the 75 and 150 mg/kg doses and for the CL criterion only at 150 mg/kg.

Dose-response curves for CNS depression also reveal statistically significant tolerance (reduced TDP scores) only at the 150 mg/kg at the $P<.05$ level for one-tailed t-test.

(b) Functional (blood level-defined) Tolerance: To evaluate functional tolerance as a function of blood levels of active drug, scattergrams of seizure thresholds were plotted against the total benzodiazepine blood level, the chlordiazepoxide level and the nor-chlordiazepoxide level on a double log plot. Regression lines were calculated by the method of least squares for the acute and for all the chronic data and the slopes compared by t-test. The results of these analyses were most revealing. For the acute drug exposures, blood levels are about 60% chlordiazepoxide, 40% nor-chlordiazepoxide and 0% demoxepam independent of dose. For the chronic, blood levels were about 25% chlordiazepoxide, 70% nor-chlordiazepoxide and 6% demoxepam again independent of dose. Because of the qualitative difference in active drug/metabolite profile, total benzodiazepine blood level plots provided the most balanced indicator of drug load to relate acute vs. chronic flurothyl scores. Maximal functional tolerance was estimated from the maximum potency shift for total benzodiazepine blood levels at the highest chronic dose and was 3.9 for MJ and 2.6 for CL criteria. The slopes of the acute vs. chronic regression lines were also significantly different by t-test for MJ (P<.001) and CL (P<.05) criteria.

Comparable plots of TDP scores versus total benzodiazepine blood levels revealed a potency shift of 3.8 for maximal functional tolerance. However, variability for the TDP score response was considerably greater than for anticonvulsant activity and the difference in slope between acute and chronic LDR regression lines was not significant (P>.10).

(c) Flurothyl Seizure Thresholds in Withdrawal: An analysis of HFE thresholds in chronic and withdrawal group controls revealed a high significant (P<.001) test-test interaction. Seizure thresholds in withdrawal were elevated about 60% for MJ and 80% for CL criteria. Despite this profound confounding influence, the flurothyl threshold for the CL criterion only was significantly (P<.02) reduced (by 8.9%) for the 75 mg/kg dose (see Fig. 10). This chlordiazepoxide dose was also the dose that was associated with the most intense spontaneous withdrawal immediately prior to flurothyl testing.

Acute Dependence Study

RO 15-1788 produced a significant anticonvulsant effect in controls. Despite this effect, seizure thresholds were dramatically reduced by 22.9% for MJ and 20.6% for CL criteria in the acutely dependent rats during preciptated withdrawal.

DISCUSSION

Like physical dependence, the results of this study demonstrate that chronic benzodiazepine anticonvulsant tolerance is a dose and blood-level dependent phenomenon. On the basis of chronic dose, tolerance for the three criteria of MJ, CL and TDP was evident only at the 150 mg/kg dose. At lower doses, tolerance developed covertly and in a more graded fashion as demonstrated by blood-level-response curve analysis. Functional tolerance was evidenced by a non-parallel shift of the blood-level response curve to a more shallow slope compared to acute suggesting a loss of maximal efficacy. However, there was no apparent ceiling to the acute or chronic, dose or blood level reponse curves so that perhaps heroic escalations of dose would have restored the initial (acute) response maxima obtained. Chronic anticonvulsant tolerance was not only dose-dependent but response dependent. It developed maximally for the highest dose and for the MJ criterion. Since the magnitude of maximal tolerance for TDP mirrored the magnitude of maximal tolerance for the MJ anticonvulsant criterion, the escalation of dose clinically to compensate for tolerance might create a no-win situation in terms of patient benefit coupled with an increased risk of physical dependence. It is noteworthy that chronic anticonvulsant tolerance against flurothyl was associated with physical dependence.

Flurothyl seizure testing during peak withdrawal from chronic chlordiazepoxide revealed a pro-convulsant change of 8.9% compared to controls. However, considering the flurothyl test-test interaction demonstrated in the water treated controls between the second and third seizure tests conducted at either 3 or 6 day intervals, an 8.9% proconvulsant action may represent only the tip of the iceburg. Moreover, the apparent 60-80% anticonvulsant effect of the test-test interaction could have neutralized a proconvulsant action of equal magnitude but opposite direction. Flurothyl test-test interaction would also explain the anomaly that a single dose of chlordiazepoxide seems to produce more proconvulsant change in withdrawal than does 5 weeks of treatment (70 doses). Clearly, spontaneous withdrawal and Ro 17-1788 precipitated withdrawal seizure threshold changes can not be fairly compared until both have been studied in acute or chronic dependence models.

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The Discriminative Stimulus Properties of Midazolam in Pigeons

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The discriminative stimulus (DS) properties of benzodiazepines have been studied extensively in rats (e.g., Colpaert et al., 1976; Shannon and Herling, 1983; Garcha et al., 1985) and to a lesser extent in primates (e.g., Ator and Griffiths, 1983, 1986; Spealman, 1985) and pigeons (Jarbe and McMillan, 1983; de la Garza et al., 1987). In general, drugs that do not bind to benzodiazepine-recognition sites do not have DS effects similar to benzodiazepines. However, the barbiturates, particularly pentobarbital, are exceptions in that there is cross-substitution between benzodiazepines and barbiturates. For instance, in pigeons trained to discriminate oxazepam (4.0 mg/kg) from saline, both benzodiazepines and barbiturates substituted for oxazepam as DS (de la Garza et al., 1987). A similar overlap between benzodiazepines and barbiturates has been demonstrated in other experiments which have evaluated the DS properties of anxiolytics in other species. For instance, pentobarbital substituted for the benzodiazepine in diazepam or chlordiazepoxide trained rats (Shannon and Herling, 1983; Colpaert et al., 1976; Haug and Gotestam, 1982). Conversely, benzodiazepines have also been reported to substitute for drug in pentobarbital trained rats (Young and Dewey, 1982). Similarly, in pigeons trained to discriminate pentobarbital from saline, both pentobarbital and diazepam substituted (Herling et al., 1980). Furthermore, the benzodiazepines diazepam and lorazepam substituted for pentobarbital in baboons (Ator and Griffiths, 1983). Thus, barbiturates and benzodiazepines has been shown to substitute for one another in various species.

Based on the ability of barbiturates and benzodiazepines to substitute for one another one might conclude that these compounds have identical DS properties. However the benzodiazepine antagonist Ro 15-1788 (Hunkeler et al., 1981) antagonizes the DS effects of benzodiazepines but not those of barbiturates (Herling and Shannon, 1982; Young and Dewey, 1982; Ator and Griffiths, 1983). This suggests that even though in many instances barbiturates and benzodiazepines share DS properties, these compounds may be differentiated from each other in terms of their mechanism of action by using selective antagonists.

Recently, other benzodiazepines have been used as DS and asymmetrical substitution between barbiturates and benzodiazepines has been demonstrated (Ator and Griffiths, 1983, 1985). For instance, baboons or rats trained to discriminate pentobarbital from saline respond on the drug lever when tested with lorazepam. However, in animals trained to discriminate lorazepam from saline, pentobarbital does not reliably substitute for lorazepam (Ator and Griffiths, 1983, 1985). Thus, lorazepam appears to be more selective as a DS than other benzodiazepines such as diazepam, oxazepam and chlordiazepoxide. Furthermore, another benzodiazepine, midazolam, has been shown to function as a DS in both rats (Garcha et al., 1985) and squirrel monkeys (Spealman, 1985) and it also appears to be more selective. For example, in squirrel monkeys trained to discriminate midazolam from saline, various sedative compounds including pentobarbital, barbital and phenobarbital failed to substitute for midazolam (Spealman, 1985).

The purpose of the present study was to determine if the DS properties of midazolam are selective in pigeons to the same extent as lorazepam and midazolam, which have been demonstrated to be selective benzodiazepines in other species. This was accomplished by training pigeons to discriminate midazolam from saline and testing various benzodiazepines as well as the barbiturates pentobarbital and phenobarbital. In most cases, the nonbenzodiazepine anxiolytic CL 218, 872 has been shown to produce DS effects similar to various benzodiazepines (de la Garza et al., 1987; McElroy and Feldman, 1982; Spealman, 1985; Ator and Griffiths, 1986). In contrast, buspirone, another nonbenzodiazepine anxiolytic, has reliably failed to share DS effects with benzodiazepines (Ator and Griffiths, 1986; Hendry et al., 1983). Therefore both CL 218, 872 and buspirone were evaluated in this study. Furthermore, to determine the pharmacological specificity of this discrimination, compounds not expected to substitute for midazolam, such as chlorpromazine, morphine, and mazindol were evaluated.

METHODS

Animals. The animals used in this study were five White Carneaux pigeons maintained at 80% of their free-feeding weights and housed individually with water and grit freely available. Purina Pigeon Checkers were provided after the session to maintain stabel weights. The pigeons were trained to discriminate 1.0 (#2779) or 3.0 mg/kg (#1490, 1859, 3315 and 7227) midazolam IM from saline and were experimentally naive at the beginning of the study.

Apparatus. The experiment was conducted in two ventilated custom-made operant chambers, each equipped with two translucent response keys which were transilluminated during the experimental session. Purina Pigeon Checkers were made available from a food magazine which was illuminated during food delivery.

Training. The discrimination training procedure used has been described in detail previously (de la Garza and Johanson, 1985). During each experimental session each pigeon was injected intramuscularly with the training dose of midazolam (1.0 or 3.0 mg/kg) or saline in a 1 ml/kg volume 10 min before the session. Following this pretreatment period, the experimental session began, signalled by the illumination of both keys and the houselight. Thirty consecutive responses (fixed-ratio 30; FR 30) on the injection appropriate key resulted in 3-sec access to food. The left key was correct after midazolam for 3 pigeons. The right key was designated correct after midazolam for the remaining pigeons. The opposite key was designated correct following saline injections. The drug-key-reinforcer relationship was maintained throughout the experiment. Responses on the incorrect key reset the fixed-ratio requirement on the correct key. Each session lasted until 50 reinforcers were delivered or until 30 minutes had elapsed, whichever occurred first.

Training sessions continued until the percent of total responses on the correct key was above 90% and the number of responses emitted on the incorrect key before the first reinforcer was delivered was less than 30 for seven consecutive sessions. The injection preceding each session was selected from a pseudorandom sequence, with the restriction that no condition would occur for three consecutive sessions.

Testing. In order to evaluate the DS properties of various benzodiazepines, sedatives and anxiolytics these compounds were administered instead of midazolam or saline during test sessions. Throughout a test session, 30 consecutive responses on either the midazolam-appropriate or saline-appropriate key resulted in food delivery. In all other respects, test sessions were identical to training sessions. Between test sessions, training sessions continued. The training drug and saline under training conditions were administered in a double alternation sequence with test sessions inserted every third session, i.e., saline, midazolam, test, midazolam, saline, test, etc. If an animal failed to meet the training criteria during a training session, further testing was postponed until the animal met these criteria on at least two consecutive training sessions.

Initially a dose-response function for midazolam was established during test sessions. Subsequently, the DS properties of various compounds were determined in a similar manner. In general, each dose of a test compound was tested once and three or four doses of each compound were tested in a mixed order. The dose-response function for each compound was completed before another compound was tested. Dose-response functions for each drug were determined in at least three pigeons. After the completion of each dose-response function, pigeons were given test sessions with either midazolam (1.0 or 3.0 mg/kg), saline, or the vehicle of the compound just tested.

Data analyses. A drug was considered to produce DS effects similar to those of midazolam if at least 80% of the total responses during the test session were emitted on the midazolam-appropriate key. The discrimination data are presented as the percentage of total responses emitted on the midazolam key for individual pigeons or as the percentage of animals tested at a particular dose that reached the 80% criterion. In addition, response rate (resp/sec) on the two keys was determined for each session. A test compound was tested until a dose was given that resulted in at least 80% of the responses on the midazolam-appropriate key or until a dose was reached which reduced response rate to at least 50% of the rate for midazolam.

Drugs. The following drugs used in this experiment were gifts: alprazolam and triazolam (The Upjohn Co., Kalamazoo, MI), morphine sulfate (National Institute on Drug Abuse), buspirone HCl (Mead Johnson Pharmaceutical Division, Evansville, IL), CL 218, 872 (Lederle Laboratories, Wayne, NJ), diazepam, mazindol, and nordiazepam (Committee on Problems of Drug Dependence, Inc.), flurazepam HCl, midazolam maleate, and nitrazepam (Hoffman-La Roche, Inc., Nutley, NJ), and lorazepam (Wyeth Laboratories, Inc., Philadelphia, PA). Chlorpromazine HCl (injectable), phenobarbital and sodium pentobarbital were purchased commercially. With the exception of CL 218,872, all injections were IM, usually in a volume of 1.0 ml/kg. When given orally, CL 218, 872 was administered PO 60 min before the session. Phenobarbital was administered IM 90 min before the session. All other drugs were administered IM 10 minutes before the session.

RESULTS

Control performances Sessions to criteria, determined from the first day when both keys were availiable required between 15 and 32 sessions (mean = 25). After the initial training phase, all five pigeons responded above 90% on the correct key throughout most of the experiment. Throughout the course of this study test sessions of saline and midazolam were conducted and the rate of responding across the five pigeons ranged from 0.91 to 3.43 resp/sec following saline and from 0.33 to 2.22 resp/sec following the training dose of midazolam. Midazolam produced a dose-related increase in the percentage of responses emitted on the midazolam-appropriate key in all five pigeons. At the lowest dose tested (0.3 mg/kg), less than 25% of responding occurred on the midazolam-appropriate key in all five pigeons whereas at the training dose (1.0 or 3.0 mg/kg) more than 95% of responding occurred on the midazolam-appropriate key. A higher dose (10 mg/kg) produced greater than 95% midazolam-appropriate responding in the 3 pigeons tested. Midazolam also produced a dose-related decrease in response rate.

Benzodiazepines. Alprazolam, diazepam, flurazepam, lorazepam, midazolam, nitrazepam, nordiazepam, and triazolam produced midazolam-appropriate responding in a dose-dependent manner in all pigeons tested. These results are shown in Figure 1 as the percentage of animals distributing at least 80% of total responses on the midazolam-appropriate key as a function of dose. At the highest dose(s) tested, midazolam-appropriate responding was well above the 80% criterion in each pigeon.

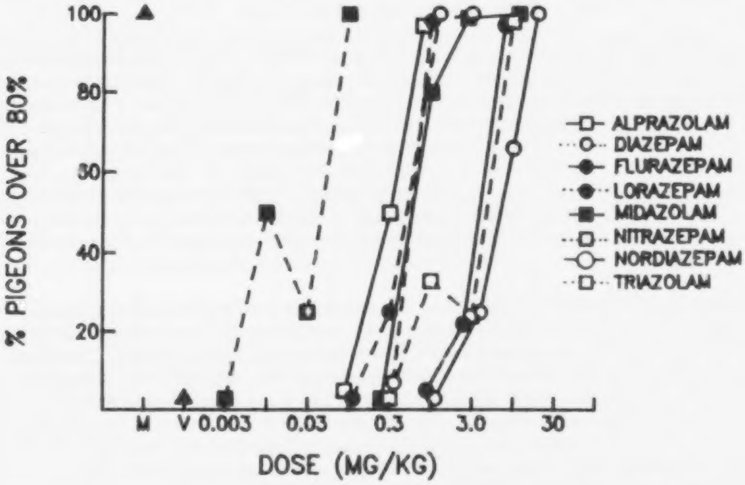

Figure 1. The percentage of midazolam-trained pigeons responding at least 80% on the midazolam-appropriate key as a function of dose during substitution test sessions. The points above midazolam (M) and vehicle (V) are the means of all midazolam and vehicle test sessions, respectively obtained during the determination of each dose-response function.

There were differences in relative potency among these benzodiazepines in terms of their ability to produce midazolam-appropriate responding. The lowest dose which produced over 80% midazolam-appropriate responding in all pigeons was 0.1 mg/kg for triazolam, 1.0 mg/kg for alprazolam, diazepam and lorazepam, 3.0 mg/kg for midazolam, 10 mg/kg for flurazepam and nitrazepam, and 17 mg/kg for nordiazepam, an acitve metabolite of diazepam.

Sedatives and nonbenzodiazepine anxiolytics. Two barbiturates, phenobarbital and pentobarbital, were also tested. These results are shown for individual pigeons in Figure 2. Phenobarbital produced greater than 80% midazolam-appropriate responding at 30 mg/kg for two pigeons and at 56 mg/kg for a third pigeon. However, phenobarbital failed to produce greater than 36% midazolam-appropriate responding in another pigeon. In contrast, pentobarbital produced greater than 80% midazolam-appropriate responding at 10 mg/kg in two pigeons and failed to substitute for midazolam in the other three pigeons tested. When the dose-response function for pentobarbital was redetermined in four pigeons, pentobarbital failed to produce 80% midazolam-appropriate responding in any them (data not shown).

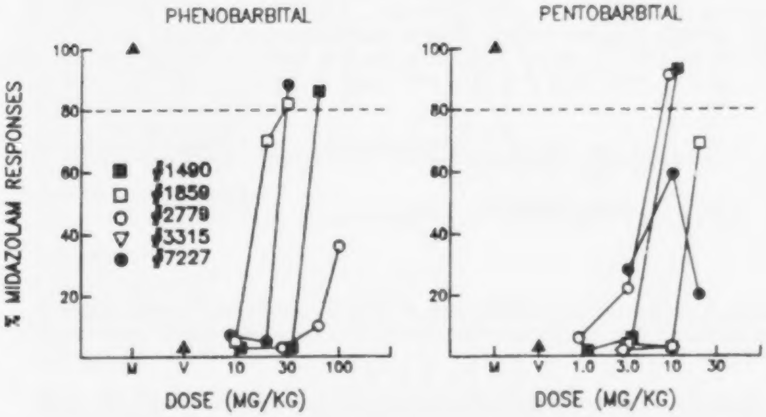

Figure 2. Effects of phenobarbital and pentobarbital in pigeons trained to discriminate 1.0 or 3.0 mg/kg midazolam from saline. The percent of midazolam-appropriate responding is shown as a function of dose during substitution tests for individual pigeons. The points above midazolam (M) and vehicle (V) are the means of all midazolam and vehicle test sessions, respectively obtained during the determination of each dose-response function.

In addition to testing benzodiazepines, other drugs used as anxiolytics were also investigated. CL 218,872 was administered both IM with a 10 min pretreatment time and PO with a 60 min pretreatment time (Figure 3). When CL 218,872 was administered IM in three pigeons it failed to produce 80% midazolam-appropriate responding in any pigeon tested. In one pigeon, 10 mg/kg CL 218,872 produced a maximum of 76% midazolam-appropriate responding. However, midazolam-appropriate responding never exceeded 50% in the other two pigeons.

Subsequently, CL 218,872 was administered orally with a 60 min pretreatment in four pigeons. CL 218,872 produced 80% midazolam-appropriate responding in only one pigeon at 10 mg/kg. In the other three pigeons CL 218,872 produced greater than 50% midazolam-appropriate responding with a maximum of 76% midazolam-appropriate responding at 30 mg/kg CL 218,872 in one pigeon. Buspirone (0.3 - 10 mg/kg), another nonbenzodiazepine anxiolytic, failed to produce midazolam-appropriate responding (less than 1%) up to doses that suppressed responding in any pigeon tested (data not shown).

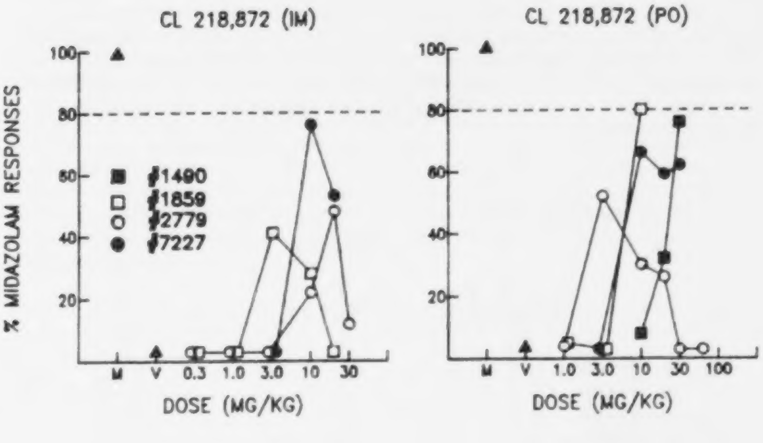

Figure 3. Effects of CL 218,872 administered IM and PO in pigeons trained to discriminate 1.0 or 3.0 mg/kg midazolam from saline. Other details as described for Figure 2.

Compounds from other pharmacological classes. Several compounds from different pharmacological classes including anorectics, morphine, and an antipsychotic were tested. The anorectic mazindol (0.01 to 3.0 mg/kg) and the antipsychotic chlorpromazine (10 to 100 mg/kg) failed to produce responding on the midazolam-appropriate key above 2% for any individual pigeon tested up to doses that substantially decreased response rate. Morphine failed to produce any midazolam-appropriate responding in three pigeons. However, in one pigeon, 3.0 mg/kg morphine produced 78% midazolam-appropriate responding but a higher dose (10 mg/kg) only produced 18% midazolam-appropriate responding and substantially reduced response rate.

DISCUSSION

The results of the present study demonstrate that midazolam is an effective DS in the pigeon and that its DS effects are pharmacologically specific. The only compounds that reliably substituted for midazolam, i.e., produced greater than 80% midazolam-appropriate responding in all pigeons tested, were other benzodiazepines. However, phenobarbital did substitute for midazolam in three of four pigeons tested. In contrast, pentobarbital failed to consistently substitute for midazolam. In previous studies CL 218,872 has been shown to substitute for benzodiazepines including midazolam in squirrel monkeys (Spealman, 1985), oxazepam in pigeons (de la Garza et al., 1987) and lorazepam in baboons and rats (Ator and Griffiths, 1986). The results from the present study conflict with previous studies in that CL 218,872 did not reliably substitute for midazolam when administered either IM or orally. However, similar findings were demonstrated in lorazepam trained rats when CL 218, 872 was administered IP; lorazepam-appropriate responding never exceeded 75% (Ator and Griffiths, 1986). In contrast, the failure of buspirone to substitute for midazolam in the present study confirms the results of other studies (Ator and Griffiths, 1986; Spealman, 1985). In addition, drugs from other pharmacological classes failed to substitute for midazolam.

In summary, the results of this study indicate that the DS properties of midazolam are not based on properties common to many CNS depressants such as their hypnotic, muscle relaxant, anticonvulsant and antianxiety properties. Similar results have been demonstrated with lorazepam (Ator and Griffiths, 1986) and midazolam trained animals (Spealman, 1985; Garcha et al., 1985). Therefore, the results of this study are consistent in that the DS effects of midazolam are more selective to benzodiazepines than other benzodiazepines such as diazepam. The ability of midazolam to serve as a selective DS in the pigeon demonstrates the reliability of using pigeons in drug discrimination procedures and thereby providing species comparisons.

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Alcohol Effects on LH and FSH in Recently Ovariectomized Female Monkeys

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INTRODUCTION

Very little is known about the effects of alcohol on anterior pituitary hormones in post-menopausal women or in ovariectomized animal models and the available data are conflicting (see Gavaler 1986 for review). Clinical studies of chronic alcoholic post-menopausal women during sobriety showed that pituitary responsivity to synthetic LHRH stimulation did not differ significantly from non-alcoholic controls (Hugues *et al.*, 1978). A single acute dose of alcohol also did not suppress LH in five normal post-menopausal women (Mendelson *et al.*, 1985). However, in the ovariectomized rat, acute alcohol administration (3.0 g/kg I.G.) significantly lowered LH levels and reduced LH pulse frequency during the second hour after alcohol administration (Dees et al., 1985). Alcohol did not significantly alter FSH secretory patterns, but prolactin levels were elevated during the first hour after alcohol administration. When synthetic LHRH (25 ng i.v.) was given 140 minutes after alcohol or saline administration, LH increased significantly in both groups. These data were interpreted to suggest that the alcohol-induced suppression of basal LH levels probably reflected a disruption in the pulsatile release of hypothalamic LHRH and that there may be separate hypothalamic regulatory mechanisms for control of FSH and LH (Dees *et al.*, 1985; McCann *et al.*, 1983).

In our previous studies of chronically ovariectomized female rhesus monkeys, alcohol administration was followed by a nonsignificant decrease in LH and no change in FSH over 120 minutes (Mello *et al.*, 1986a, 1987). Whereas, after synthetic LHRH administration, a significant alcohol dose-dependent increase in LH was observed (Mello *et al.*, 1986a, 1987). FSH was also higher after alcohol (3.5 g/kg) than sucrose control administration but increases were not dose-dependent (Mello *et al.*, 1986a).

In rhesus monkey, ovariectomy is followed by a rapid increase in anterior pituitary hormone levels and a circhoral pattern of LH activity (Atkinson *et al.*, 1970; Dierschke *et al.*, 1970). An initial elevation in LH following abrupt removal of ovarian steroid feedback eventually declines to a stable level between 100 - 200 ng/ml. It is not known if the relative sensitivity of the hypothalamic-pituitary axis to alcohol's effects is different in recently ovariectomized females and chronic castrates. The primary goal of the present study was to examine the acute effects of alcohol (2.5 - 3.5 g/kg) on basal levels of LH and FSH in recently ovariectomized female rhesus monkeys. LH and FSH pulse frequency and amplitude were also analyzed.

METHODS

Subjects: Six bilaterally ovariectomized female rhesus monkeys (6.2 ± 0.42 kg) were studied. Each monkey was first exposed to a single dose of alcohol (2.5 g/kg) within 18 ± 2.1 days after ovariectomy, and the last study was completed within 40 to 70 days following ovariectomy ($\bar{X}$ = 52 ± 4.4 days). Two monkeys were alcohol naive and the others had a history of low dose alcohol self-administration but had been alcohol free for 5 to 19 months prior to these studies. Monkeys were maintained on ad lib food and water; monkey chow was supplemented daily with fresh fruit, vegetables and multiple vitamins. A 12-hour light-dark cycle (7:00 a.m. to 7:00 p.m.) was in effect. Animal maintenance and research was conducted in accordance with recommendations by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facility is licensed by the Department of Agriculture. The health of the monkeys was periodically monitored by a consultant veterinarian from the New England Regional Primate Research Center.

Sequence of Conditions: The acute effects of alcohol and sucrose control solutions on basal levels of LH and FSH were evaluated in the same subjects under identical conditions. Basal levels of LH and FSH were measured for 180 minutes before alcohol or sucrose was administered and for 360 minutes following alcohol or sucrose administration. Integrated plasma samples were collected at 20 minute intervals throughout the study. Samples for blood alcohol analysis were collected every 40 minutes beginning with the second post-alcohol sample. Details of the integrated plasma sample collection procedures (Bree et al., 1982) and the radioimmunoassay procedures for LH and FSH analysis have been published (Mello et al., 1986a). The LH assay sensitivity was 6.5 ng/ml. Intra- and interassay coefficients of variation were 5.4% and 10.6% respectively. The FSH assay sensitivity was 1.3 ng/ml and the intra- and interassay coefficients of variation were 6.4% and 9.0% respectively. The plasma alcohol assay sensitivity was 16.2 mg/dl. Intra- and interassay coefficients of variation were 3.0% and 4.5% respectively.

Alcohol Administration: Alcohol (2.5, 3.0 and 3.5 g/kg) prepared in a 25% solution was administered through a pediatric grade nasogastric tube. Alcohol effects were compared with a sucrose control solution isocalorically equivalent to 2.5 g/kg of alcohol. Monkeys were fasted for 18 to 20 hours to ensure uniform absorption of alcohol. Alcohol and sucrose control solutions were given in an irregular order in the following sequence: 2.5 g/kg alcohol, 3.5 g/kg alcohol, sucrose control and 3.0 g/kg alcohol.

RESULTS

LH Levels after Alcohol/Sucrose: ANOVA showed a significant effect of dose ($P < .001$) and of condition (pre vs post-alcohol or sucrose) ($P < .001$) on LH. Basal LH averaged 270 ± 20 ng/ml before sucrose control administration and decreased significantly to an average of 216 ± 8 ng/ml over the next 6 hours of observation ($P < .05$). A one way ANOVA for repeated measures showed a significant main effect ($P < .04$). There were no significant differences between baseline LH levels prior to sucrose and alcohol (samples 1-9).

Average LH levels also decreased significantly after administration of a moderate alcohol dose (2.5 g/kg) ($P < .01$). Baseline LH levels averaged 363 ± 46 ng/ml and decreased by 26% to 269 ± 25 ng/ml after alcohol. LH levels were significantly lower than baseline during both the ascending (samples 10-18) and the stable phase (samples 19-27) of the blood alcohol curve. Blood alcohol levels gradually increased to peak values 256 ± 16 mg/dl within 200 minutes after nasogastric intubation of 2.5 g/kg of alcohol.

Administration of a higher alcohol dose (3.0 g/kg) resulted in a significant ($P < .01$) suppression of LH. Basal LH levels averaged 223 ± 14.6 ng/ml and decreased to 174 ± 6 ng/ml after alcohol administration. LH fell significantly within 80 minutes when blood alcohol levels averaged 192 ± 14 mg/dl and remained significantly suppressed ($P < .05$, $.01$) for five hours. Peak blood alcohol levels were 289 ± 34 mg/dl within four hours after alcohol administration.

After the highest dose of alcohol studied (3.5 g/kg), blood alcohol levels increased to above 300 mg/dl within 160 minutes. Peak blood alcohol levels were 327 ± 33 mg/dl at 3 hours 20 minutes after alcohol administration. LH levels decreased from a baseline average of 229 ± 12 ng/ml to 202 ± 12 ng/ml but this difference was not statistically significant.

FSH Levels after Alcohol/Sucrose: ANOVA showed a significant main effect of dose ($P < .001$) and a significant effect of condition (pre- versus post-alcohol and sucrose) ($P < .001$) on FSH. Basal FSH levels were equivalent before sucrose/alcohol administration and ranged between 79 ± 2.1 and 89 ± 3.4 ng/ml. One way ANOVA for repeated measures showed that basal FSH levels did not change significantly after sucrose control administration.

Alcohol consistently supressed FSH. After administration of 2.5 g/kg of alcohol, basal FSH levels decreased significantly ($P < .001$). Dunnett's test showed that FSH fell significantly below baseline levels ($P < .01$) when blood alcohol levels exceeded 200 mg/dl and remained suppressed for 2 hours. After administration of 3.0 g/kg alcohol, FSH also decreased significantly below baseline ($P < .001$). FSH declined significantly within two hours after alcohol administration when blood alcohol levels exceeded 200 mg/dl and remained suppressed for 80 minutes ($P < .01$). This significant decrease in FSH occurred 40 minutes after suppression of LH. FSH also decreased significantly after administration of 3.5 g/kg of alcohol ($P < .001$). FSH fell abruptly within 2 hours after alcohol administration when blood alcohol levels averaged 266 ± 31 mg/dl. A decline in LH paralleled the initial significant decline in FSH but FSH remained significantly below baseline for 160 minutes

LH and FSH Pulse Frequency and Amplitude: Group average LH and FSH pulse frequency per hour during the three hour pre-sucrose/alcohol condition and the six hour post-sucrose/alcohol were evaluated with a computer program based on the procedures of Santen and Bardin (1973). A peak was defined as an increase of 20% or more over the immediately preceding nadir. During the pre-alcohol conditions, LH pulse frequency averaged 0.61 to 0.77 pulses per hour. This is similar to LH pulse frequency measured during the early luteal phase (0.8 pulses per hour) in normal female monkeys (Ellinwood and Norman 1983). LH pulse frequency decreased following the administration of both sucrose and alcohol. LH pulse frequency after alcohol ranged between 0.41 and 0.36 pulses per hour. LH pulse frequency after sucrose control administration was 0.47 pulses per hour. This is similar to the average LH pulse frequency measured during the early follicular phase (0.49 per hour) by Van Vugt and co-workers (1984).

FSH pulse frequency prior to sucrose or alcohol administration ranged between 0.386 and 0.50 pulses per hour. There were no significant pre vs. post differences in FSH pulses per hour and no orderly alcohol dose effects on FSH pulse frequency. We are unaware of previous reports of FSH pulse frequencies in female rhesus monkeys.

LH pulse amplitude also declined after administration of both sucrose control solution and 2.5 and 3.0 g/kg of alcohol. But after administration of 3.5 g/kg of alcohol, average LH pulse height increased. There was no systematic pattern of changes in FSH pulse amplitude across conditions.

DISCUSSION

LH after Alcohol/Sucrose: No consistent effects of alcohol on LH secretory activity were observed in this group of recently ovariectomized monkeys. Administration of 2.5 and 3.0 g/kg significantly suppressed LH but the highest alcohol dose (3.5 g/kg) had no significant effect on LH. Interpretation of these data is further complicated by the fact that LH levels were also significantly suppressed after sucrose control administration. Analysis of LH pulse frequency and amplitude also indicated a decline in LH pulses per hour after both sucrose and alcohol administration.

Consequently, it is difficult to attribute data obtained to the effects of alcohol per se.

The factors that accounted for a significant suppression of LH by sucrose control administration are unclear. It is possible that LH suppression may reflect the nonspecific stress associated with the procedure. It is well established that both alcohol and "stress" stimulate CRF (corticotropin-releasing-factor) and subsequently ACTH release which may inhibit pituitary gonadotropin secretion (Redi et al., 1986; Rivier et al., 1984, 1986). Administration of an antagonist to CRF has been shown to reverse the inhibitory effect of stress on pulsatile LH release in rats (Rivier et al., 1986). Unfortunately, ACTH was not measured in monkeys in the present study so there are no data available to directly confirm or refute the non-specific stress hypothesis.

The lack of alcohol dose-related effects on LH are concordant with previous observations in post-menopausal women (Mendelson et al., 1985) and in long term ovariectomized monkeys where basal LH and FSH levels were unchanged for the first 2 hours after alcohol administration (Mello et al., 1986a, 1987). These data in women and female rhesus monkeys are inconsistent with studies in ovariectomized rats where a single dose of alcohol (3.0 g/kg I.G.) suppressed basal levels of LH but not FSH (Dees et al., 1985). Species differences as well as several differences in methodology limit comparisons between the present study in female monkeys and previous studies in rats (Dees et al., 1985).

FSH after Alcohol/Sucrose: Both group and individual data analysis indicated that sucrose control administration had no effect on FSH but alcohol significantly depressed FSH at all doses ($P < .05 - .001$). However, no consistent alcohol dose-related effects on FSH were observed. We have previously reported a differential effect of alcohol on LH and FSH in normally cycling females during the follicular phase of the menstrual cycle after synthetic LHRH stimulation (Mello et al., 1986b). However, in long term ovariectomized females, there was no differential effect of alcohol on LHRH stimulated LH and FSH (Mello et al., 1986a). LHRH stimulated FSH increased significantly under both control and alcohol conditions and alcohol appeared to enhance LHRH stimulated LH and FSH in comparison to control conditions (Mello et al., 1986a, 1987). We interpreted the contrast between alcohol's effects on LHRH stimulated FSH in long term ovariectomized females and in normally cycling female rhesus monkeys as evidence of the importance of ovarian negative feedback on pituitary FSH secretory cell activity (Mello 1986a and b). We argued that since the ovarian peptide, inhibin, has been shown to suppress FSH without affecting LH in several species under many conditions (Channing et al., 1985; McCann et al., 1983) alcohol may have enhanced the effects of inhibin in normally cycling females (Mello et al., 1986a and b).

Alcohol's suppression of FSH but not LH in the recently ovariectomized females described in this study obviously cannot be attributed to ovarian inhibin. It is not clear if these differences in alcohol effects on FSH between recently and chronically ovariectomized monkeys reflect changes in the relative sensitivity of the hypothalamic-pituitary axis as a function of time after ovariectomy or are more parsimoniously attributed to procedural differences, i.e. measures of basal hormone levels in the present study and LHRH stimulated gonadotropin patterns in our previous report (Mello et al., 1986a). Our current data are consistent with the hypothesis that there is a separate hypothalamic regulatory factor (FSH-RH) which controls FSH separately from LHRH control of LH (McCann et al., 1983). These observations are also consistent with previous reports that acute alcohol administration was associated with a significant decrease in FSH over time in human females (Valimaki et al., 1983). Studies are now in progress to compare the effects of alcohol on LH and FSH pulse frequency in recently and chronically ovariectomized females and to determine if the relative sensitivity of the hypothalamic-pituitary axis to alcohol effects changes as a function of time after ovariectomy.

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Adrenal Enkephalin Biosynthesis Regulated by Glucocorticoid

C. Inturrisi, S. Franklin, J. Shapiro, S. Calvano and B. Yoburn

Denervation of the rat adrenal results in a 10 to 20-fold increase in enkephalin-containing EC peptides 4 days after surgery. This denervation-induced rise in medullary EC peptides is blocked by hypophysectomy (hypox) and partially reinstated by dexamethasone or ACTH treatment. Culture of adrenal medullary explants (glands) for 4 days in serum-free medium without dexamethasone resulted in a 6-fold increase in EC peptides in glands from hypox rats while the addition of dexamethasone (10-5M) produced a 29 fold increase. These results indicate that a loss of depolarizing stimuli produced by denervation in vivo or explantation *in vitro* initiates a pretranslational event which requires glucocorticoid for the full expression of EC peptide biosynthesis.

INTRODUCTION

Although the physiological function of the adrenal EC peptides is only now emerging, the EC peptides are co-released with the catecholamines (CAs) (Livett *et al*., 1981) and may be involved in mediating some forms of stress-induced analgesia (Lewis *et al*., 1982). When depolarizing influences are removed from the adrenal medulla *in vivo* by surgical denervation or by pharmacological denervation by the use of the ganglionic blocking agent, chlorisondamine, there is a large increase in EC peptide levels that reaches a peak at 4 days after treatment (Fig. 1). Also, explanation of rat adrenal medulla in culture for 4 days produces an increase similar to that seen following denervation *in vivo*. Furthermore, inclusion of potassium ions (Fig. 1) in the culture medium prevents the culture-induced increase in EC peptides, presumably by maintaining depolarizing conditions (Bohn *et al*., 1983, Fleminger *et al*., 1984a, Fleminger *et al*., 1984b, Inturrisi *et al*., 1987, LaGamma *et al*., 1984, LaGamma *et al*., 1985, Lewis *et al*., 1982, Yoburn *et al*., 1987). The major species of newly appearing EC peptide is represented by the intact precursor proenkephalin (Fleminger *et al*., 1984a, Fleminger *et al*., 1984b).

Because of the critical role played by the pituitary-adrenal axis in stress, we have examined the influence of this system and of glucocorticoid in the regulation of the rise in EC peptides which occurs when the rat adrenal medulla is denervated *in vivo* or placed in culture.

MATERIALS AND METHODS

Studies of glands *in vitro* were conducted in male Sprague-Dawley rats nine days *following* hypophysectomy so that the adrenal cortex would have atrophied and endogenous corticosterone levels would be minimal. In some *in vivo* studies, nine days following hypophysectomy, the *left adrenal* medulla was denervated and the contralateral gland served as the intact, unoperated control. In separate studies, rats were hypophysectomized and 48 hrs later injected sc once per day with 1 mg/kg dexamethasone (N=6), 4 units adrenocorticotrophic hormone (ACTH) (N=7) or 1 ml/kg vehicle (N=9) for eleven days. On the eighth injection day, the left adrenal medulla was denervated in all rats. Four days following denervation (13 days following hypophysectomy), rats were sacrificed and the left and right medullae collected.

Rat adrenal medullary explants were cultered for 4 days in RPMI-1640 (serum free medium) at 37° C in an atmosphere of 95% air and 5% CO2 at nearly 100% relative humidity.

Each adrenal medulla was assayed for EC peptides as described previously (Fleminger *et al.*, 1984b). EC peptides were determined by radioimmunoassay (RIA) before (Free) and after (Total) treatment with trypsin and carboxypeptidase B. The enzymatic digestion was used to release [Met]enkephalin from precursor peptides and allowed measurement of the Total [Met]enkephalin content of the gland. The lower limit of sensitivity for the EC peptide RIA is 5.6 pg and the interassay coefficient of variation for 12 consecutive assay averaged 6.6%. Corticosterone levels in serum and the glands were measured by use of RIA (Keith *et al.*, 1978). Average assay sensitivity was 12.5 ± 2 ng/ml and the mean interassay coefficient of variation for 4 consecutive assays was 9.6%.

Data were analyzed by analysis of variance and t-tests. Post hoc comparison were made with the one and two-tailed t-test using the error term from the preceding analysis of variance.

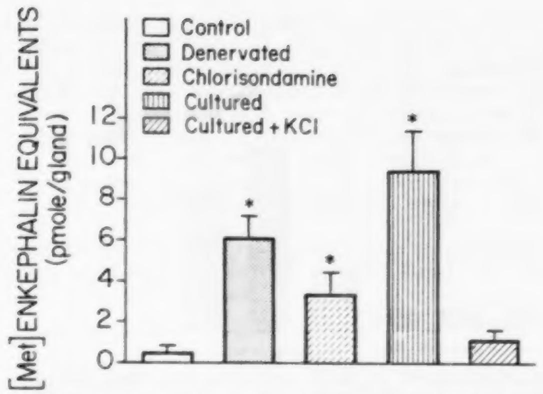

FIGURE 1. Surgical denervation, pharmacological denervation and culture increase rat adrenal medullary EC peptides. Medullae were collected 4 days after surgical denervation, treatment with chlorisondamine (5 mg/kg) and atropine (2 mg/kg) sc, twice a day for 4 days or explantation for 4 days in a medium of RPMI-1640 plus 10% horse serum, 5% fetal calf serum and dexamethasone 10^{-5}M in the presence or absence of 55 mM KCl. *indicates significantly different (p<.01) from control (untreated) animals.

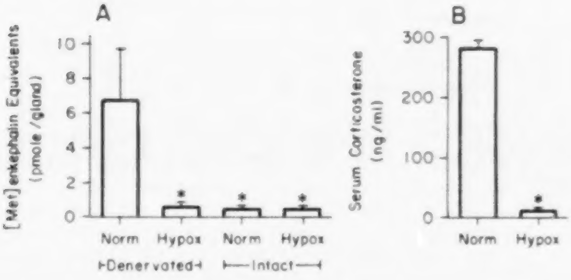

FIGURE 2. Hypophysectomy prevents the denervation-induced increase in EC peptides ([Met]-enkephalin equivalents) in rat adrenal medullae. *indicates significantly different (p<.01) from Normal-Denervated value B. Serum corticosterone levels in hypophysectomized (Hypox) and Normal rats. *indicates significantly different (p<.001) from normals.

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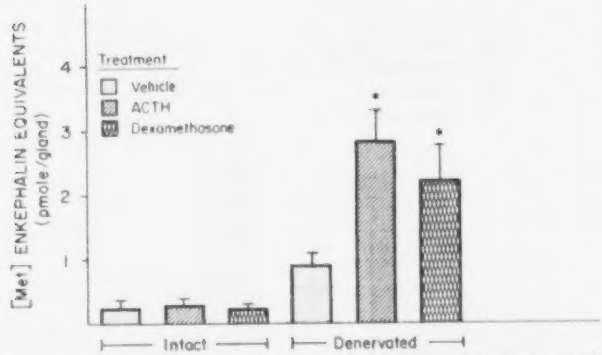

FIGURE 3. Dexamethadone or ACTH restores the denervation-induced increase in adrenal medullary EC peptides ([Met]-Enkephalin equivalents) in hypophysectomized (Hypox) rats. *indicates significantly different ($p < .01$) from intact value.

TABLE 1. Adrenal Medullary EC Peptides are Increased Maximally by Culture for 4 Days in Serum Free Medium with Dexamethasone.

Type of Gland	Days in Culture	Additions	pmole/gld	(+SE)
sham	0	none	0.4	(0.1)
sham	4	none	* 5.0	(0.7)
sham	4	Dexamethasone, 10^{-5}M	*+ 9.4	(1.1)
hypox	0	none	0.3	(0.04)
hypox	4	none	* 1.9	(0.3)
hypox	4	Dexamethasone, 10^{-5}M	*+ 8.6	(0.9)

* significantly different from corresponding 0 day group ($p < .01$)
+ significantly different from corresponding 4 day group without Dex ($p < .05$)

RESULTS

Hypophysectomy completely blocked the denervation-induced increase in EC peptides (Fig. 2A) and produced virtually a complete loss of handling-stimulated corticosterone release (Fig. 2B). Hypophysectomy had no effect on the low levels of EC peptides present in the intact right gland (Fig. 2A). As shown in Fig. 3 we could partially reinstate the denervation-induced increase in adrenal EC peptides in the hypophysectomized rat by treating with ACTH or dexamethasone.

To more definitively examine the role of glucocorticoid, sham and hypox glands were placed in serum free medium for 4 days and compared with control glands obtained at the time of sacrifice. In the absence of dexamethasone, EC peptides increased significantly in sham and hypox glands (Table 1). The average increase for all experiments was 13 fold for sham and 6 fold for hypox glands. However, the addition of dexamethasone 10-5M produced a much greater increase in EC peptides in sham and hypox glands which averaged, for all experiments, 24 fold for sham and 29 fold for hypox glands. In serum free medium hypox glands show a concentration dependent increase in EC peptides with the ED50 for dexamethasone equal to 5.7×10^{-7}M (Inturrisi et al., 1987).

The nonglucocorticoid steroids, progesterone, testosterone and deoxycorticosterone did not alter EC peptide levels in hypox glands (unpublished observations).

DISCUSSION

The present report demonstrates that glucocorticoid is responsible for most of the dramatic increase in rat adrenal medullary EC peptides that occurs when depolarizing stimuli are removed by denervation or explantation. Hypophysectomy blocked the denervation-induced increase by eliminating ACTH and indirectly reducing corticosterone levels at the gland. Both ACTH, by preserving adrenal cortical function, and dexamethasone, acting directly on the gland, allowed expression in hypophysectomized rats of the denervation-induced increase in EC peptides. However, the doses of dexamethasone and ACTH chosen were apparently not optimal for full restoration of the denervation effect in hypophysectomized rats. Thus, taken together, these results show that glucocorticoid but not ACTH is required for EC peptide biosynthesis in the denervated rat adrenal.

The results obtained in culture support and extend the in vivo observations. The stimulatory action of glucocorticoids on EC peptides was not shared by steroids devoid of glucocorticoid activity. When glands from sham rats are placed in glucocorticoid free medium, EC peptide levels are significantly increased but become dependent upon the addition of dexamethasone for full expression. Glands from hypox rats were almost entirely dependent upon dexamethasone for EC peptide biosynthesis (Table 1). Medullae from sham rats contained higher levels (27 ng/gland) of corticosterone while in isolated hypox medullae, the corticosterone levels had decreased to less than 1% of sham glands and were at the limits of sensitivity of the assay. Thus, in order to completely separate the contribution of glucocorticoids, glands from hypox animals must be employed.

In rat adrenal medullary explants, the increased levels of EC peptides are dependent on protein synthesis and are preceded by an increase in preproenkephalin mRNA (LaGamma et al., 1985). Thus the effects of adrenal denervation or explantation on EC peptides appear to be mediated at a genomic level. Glucocorticoids are known to modulate the transcription of specific genes. In this regard a consensus DNA binding site for the glucocorticoid-receptor complex on several responsive genes has been identified as the core sequence 5' TGTC/TCT 3' (Hollenberg et al., 1985) and the rat proenkephalin gene includes this core sequence (Rosen et al., 1984).

The physiological response of the adrenal medulla to stress includes increases in both transsynaptic impulse activity and glucocorticoid levels. The recognition that both of these factors are capable of regulating EC peptides as well as catecholamines adds significantly to our understanding of the complexity involved in the physiological response to stress and may point the way to new approaches to define the sites of action and potential therapeutic utility of EC peptides.

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Neuroadaptation of Rats to Kappa Agonists U-50,488 and Tifluadom

C. Murray and A. Cowan

INTRODUCTION

Analgesic pharmacologists have long been interested in the behavioral disturbances that appear after abrupt withdrawal of opioids from chronically dosed animals, or after challenging these animals with narcotic antagonists such as naloxone. Agonists at *mu* opioid receptors have been used extensively in this type of research. Agonists showing selectivity for *kappa* opioid receptors have only recently become available for study. The current view is that agents of the latter class (e.g., U-50,488) are not associated with a morphine-like withdrawal syndrome, at least in mice and rats [Tang and Collins, 1985; Cowan *et al.*, 1986]. This belief has helped to reinforce the concept of developing selective, nonbenzomorphan *kappa* agonists (e.g., spiradoline) as clinically useful analgesics.

The question arises, however, as to whether or not rodents demonstrate any neuroadaptation in response to sustained administration of a *kappa* agonist such as U-50,488H (U50; Upjohn) or (±)-tifluadom (TIF; Kali-Chemie). Second, if neuroadaptation occurs, to what extent can it be unveiled by traditional approaches such as abrupt withdrawal or challenge with opioid antagonists? Third, if such adaptation can be revealed, and is accepted as evidence of physical dependence of the *kappa* type, does tolerance develop in parallel? These issues are addressed in the present report.

MATERIALS AND METHODS

Animals. Male Sprague Dawley albino rats (Zivic-Miller Laboratories, 200-275 g), male ICR mice (Temple University Skin and Cancer Hospital, 25-35 g), and male Hartley guinea pigs (Charles River, 250-350 g) were used. They were housed 5 per cage with food and water available *ad libitum*. A 12 hr light/dark cycle was maintained (lights on at 7:00 a.m.; all experiments were carried out during the light phase).

Chronic U50 administration. Method 1. Osmotic minipumps (model 2001, Alzet) were implanted s.c. in rats, mice and guinea pigs. These pumps had a fill volume of 237 ± 14 (S.D.) μl and released U50 at a rate of 18 μg/hr. Antagonists were given 4 d after implantation. Method 2: Rats were injected s.c. with U50 at 8:30 a.m. and 8:30 p.m. over 5 d. On the fifth day, only the morning injection was given. (In the case of the abrupt withdrawal experiment, this ninth injection was omitted.) Doses were: day 1 - 10 mg/kg; day 2 - 20 mg/kg; days 3 to 5 - 40 mg/kg. A similar experiment was done in which the doses were ten times lower. This procedure was also carried out with (±)-TIF using doses of 3.5 and 0.35, 7 and 0.7, and 14 and 1.4 mg/kg, s.c., respectively. If antagonists were to be given, they were administered 4 h after the last agonist injection. For both methods, on day 4 the animals were acclimated to their plexiglass observation cages in a constant temperature room (20 ± 1 °C) for 1 hr, then trained to have their weights and rectal temperatures taken with a minimum of stress. On day 5, this procedure was repeated (after the last agonist dose for method 2) before baseline readings and antagonist injections.

Endpoints. (a) During abrupt withdrawal, the following behaviors (presumed to be evidence of physical dependence) were counted for one 15 min and four 30 min observation periods starting at 10:00 a.m. on day 5: licking of the mouth/tongue protrusions; chewing movements; gnawing of the bedding and/or feces; repetitive sniffing of the bedding; yawning; coughing; rearing; "wet dog" shakes. (b) After antagonists: change in rectal temperature from t=0 (just before antagonist injection) to t=60 min post-injection; weight loss over same time span, "wet dog" shakes – counted for 30 min post-injection; rearing – counted for 30 min post-injection; other behaviors as listed in Tables 3-5; general behavior/affect – observed for 60 min post-injection, checked periodically over the following 5 hr.

Abrupt withdrawal study. Ten rats were given the course of U50 injections described above; in parallel, 10 rats were given saline injections. Behavioral observations commenced at 8:30 a.m. of day 5, using the endpoints outlined above.

Antagonist studies. (a) Osmotic minipumps. Five mice and 4 guinea pigs were each implanted with minipumps containing U50; 4 days later they were injected with buprenorphine (1 mg/kg, s.c., NIDA) and observed for signs of abstinence. Rats (n=20 overall, n=5 per group) were similarly implanted and on day 4 received buprenorphine (0.1, 1, and 10 mg/kg, s.c.) or naloxone (3 mg/kg, s.c., Endo). In parallel, 20 rats were sham operated and received the same doses of buprenorphine; the fourth group was injected with saline as a baseline control for the weight and temperature data. (b) Repeated injections. Using the schedules detailed above, rats (n=4–10 per group) were injected with U50, TIF, or saline over 5 days. On day 5, buprenorphine (high and low dose U50/TIF regimens) or naloxone (high dose U50 regimen only) were injected s.c. and the animals were observed for behavioral signs of abstinence; temperature and weight changes were also recorded. The antagonist doses were 1 mg/kg of buprenorphine and 3 mg/kg of naloxone, respectively.

Tolerance studies. U50 was administered to rats at 18 mg/kg, s.c. After 30 min, the animals were assessed for antinociception using the hypertonic saline (4% NaCl, i.p.) writhing test [Collier and Schneider, 1969]. Only the animals in which this dose of U50 was analgetic were selected. One group (n=16) was implanted with minipumps containing U50 (see above); a second control group (n=14) was sham-operated. Four days later the same dose of U50 was evaluated in these 30 rats. This experiment was also carried out via the course of U50 injections described above, save that an 18 mg/kg test dose took the place of the first 10 mg/kg injection (n=10 each for U50 and parallel vehicle injections). The 18 mg/kg dose for the test on day 5 was given 3 hr after the last 40 mg/kg dose. We defined tolerance as the inability of the same dose of U50 to produce analgesia in an animal after a chronic exposure toU50.

RESULTS AND DISCUSSION

The data summarized in Table 1 set the scene for the present range of experiments. Abrupt withdrawal from U50 in rats triggered a higher incidence of dopaminergic/muscarinic behaviors than was seen with vehicle control animals. This set of behaviors was not observed when U50 was delivered from minipumps and the rats challenged with naloxone or buprenorphine, an analgesic with *kappa* antagonist properties *in vivo* [Richards and Sadée, 1985; D.E. Gmerek, personal communication]. Signs of morphine-like abstinence were also absent in these rats (Table 2), as well as in mice and guinea pigs receiving U50 from minipumps (data not shown).

Of great interest was our finding that dopaminergic/muscarinic behaviors (oral stereotypies - gnawing, tongue protrusion, cage licking; Figure 2) were precipitated by buprenorphine challenge in rats receiving injections of U50. This observation was unexpected since dogma would have it that a continuous supply of agonist to opioid receptors is more likely to provoke neuroadaptation than twice-daily injections. Buprenorphine-induced oral stereotypies were dependent on the doses of U50 and TIF (Tables 3 and 4), had an onset of about 5 min, and lasted for more than 4 hr. As part of a syndrome, these rats also demonstrated a high degree of general behavioral activation (e.g., agitation, rearing) vs. controls. Similarly, naloxone precipitated behavioral changes in rats receiving U50 by injection but not from minipumps. In this case, increased yawning and writhing were noted (Table 5) whereas marked changes in temperature and weight did not occur. Importantly, the behaviors mentioned in this paragraph were not observed when buprenorphine (1 mg/kg), naloxone (3 mg/kg) or sufentanyl (1 μg/kg) were given s.c. to rats 4 hr after a large standard dose of U50 (40 mg/kg, s.c.) (data not shown). In general, the syndrome precipitated by antagonist injection was more intense than that brought about via abrupt withdrawal.

Data from the tolerance study are presented in Figure 1. Tolerance clearly develops to the antinociceptive action of U50 in the rat hypertonic saline test when this *kappa* agonist is delivered s.c. from minipumps or via twice-daily injections.

TABLE 1. *Summary of behavioral data from abrupt withdrawal study. Values represent the mean ± S.E.M. number of episodes of the indicated behavior from the five observation periods (see Methods for details). *p<0.05, **p<0.01, Mann-Whitney U test vs. saline controls (n=10 per group; vehicle control data not shown to conserve space).*

U-50 treated	TIME FRAME (hr after last scheduled U50 dose)				
Endpoint	1.5 - 1.75	2 - 2.5	3 - 3.5	4.5 - 5	6.5 - 7
Mouth licking	**0.7 ±0.3***	**1.7 ±0.7***	**1.9 ±0.9***	**0.6 ±0.2***	**1.0 ±0.6**
Chewing	**0.2 ±0.2**	**1.2 ±0.7**	**2.6 ±0.9***	**3.3 ±1.0***	**3.1 ±1.6**
Gnawing bedding/feces	**0.5 ±0.4**	**3.3 ±1.4****	**1.6 ±0.8**	**1.7 ±0.9**	**1.4 ±1.1**
Sniffing bedding	**1.4 ±0.4***	**7.2 ±2.6***	**3.9 ±1.8**	**3.4 ±1.6**	**1.5 ±1.2**
Yawning	**0.5 ±0.3**	**0.5 ±0.2**	**1.6 ±0.5***	**2.3 ±1.0***	**1.7 ±0.6**
Coughing	**0.5 ±0.3**	**0.8 ±0.3***	**1.0 ±0.4**	**1.8 ±0.8***	**0.0 ±0.0**
Rearing	**2.1 ±1.5**	**12.1 ±5.3**	**3.9 ±2.0**	**1.7 ±1.7**	**0.5 ±0.5**
"Wet dog" shakes	**0.8 ±0.4**	**0.9 ±0.4**	**1.3 ±0.8**	**1.6 ±0.5****	**1.1 ±0.5**

TABLE 2. *Summary of precipitated abstinence data in rats given chronic U50 by osmotic minipump and subsequently challenged with buprenorphine (B) or naloxone (Nx). Data are mean values from indicated time or observation period after injection of B or Nx. Doses [in square brackets] are in mg/kg, s.c. (n=5 per group; values in parentheses are S.E.M.)*

Treatment	*Endpoint*			
	ΔT, rectal (°C, 60 min)	Δ Body weight (%, 60 min)	Rears (30 min)	WDS (30 min)
Sham - vehicle	-0.3 (0.1)	-0.98 (0.26)	1.0 (0.5)	0.2 (0.2)
Sham - B [0.1]	1.4 (0.1)	-1.46 (0.29)	2.6 (1.9)	0.2 (0.2)
Sham - B [1]	1.1 (0.3)	-1.11 (0.24)	17.8 (14.7)	1.4 (0.7)
Sham - B [10]	1.3 (0.4)	-0.69 (0.25)	1.0 (0.5)	0.2 (0.2)
U50 - B [0.1]	1.2 (0.1)	-1.31 (0.23)	8.2 (6.3)	0.6 (0.6)
U50 - B [1]	1.4 (0.2)	-0.80 (0.15)	1.0 (0.8)	0.0 (0.0)
U50 - B [10]	1.7 (0.2)	-1.92 (0.48)	11.2 (6.9)	0.0 (0.0)
U50 - Nx [3]	-0.4 (0.2)	-1.05 (0.16)	8.2 (8.0)	0.4 (0.4)

TABLE 3. *Number of rats per group showing oral stereotypy (cage licking) precipitated by buprenorphine (1 mg/kg, s.c.) after chronic dosing with U50 or saline. High dose regimen was from 10 to 40 mg/kg (s.c.); low dose regimen was from 1 to 4 mg/kg (s.c.). See Methods for details. *p<0.025, Fisher's exact test vs. saline control animals.*

Treatment	High Dose U50	Low Dose U50
Saline - buprenorphine	0/5	0/4
U50 - buprenorphine	4/5 *	1/4

TABLE 4. *Effects of buprenorphine [1 mg/kg, s.c.] in rats given chronic tifluadom [3.5 to 14 mg/kg, s.c.] and chronic vehicle injections. Values represent the mean ± S.E.M. rectal temperature change (ΔT), body weight (wt.) change, or number of episodes of the indicated behavior during the listed observation periods (times are post-antagonist injection; see Methods for details). NS = not significant vs. vehicle controls, Student t test;* *$p<0.05$, **$p<0.01$, *Mann-Whitney U test vs. vehicle controls (n = 10 per group).*

	TREATMENT			
ENDPOINT	Chronic Tifluadom		Chronic Vehicle	
Δ T, rectal (°C, 60 min)	**+ 1.3**	**± 0.2 NS**	**+ 0.9**	**± 0.2**
Δ body wt. (%, 60 min)	**- 0.63**	**± 0.16 NS**	**- 0.70**	**± 0.14**
Cage licking (30 min)	**12.3**	**± 4.4 ****	**0.0**	**± 0.0**
Sniffing bedding (30 min)	**11.5**	**± 4.4**	**9.8**	**± 3.4**
Gnawing bedding/feces (30 min)	**17.8**	**± 4.6 ***	**4.2**	**± 1.4**
Coughing (30 min)	**1.9**	**± 0.7 ***	**0.0**	**± 0.0**
Agitation (30 min)	**4.3**	**± 1.5 ***	**0.3**	**± 0.2**
Rearing (30 min)	**17.3**	**± 5.9 ***	**7.2**	**± 3.6**
Jumping (30 min)	**1.9**	**± 0.9**	**0.0**	**± 0.0**

TABLE 5. *Effects of naloxone [3 mg/kg, s.c.] in rats given chronic U50 [10 to 40 mg/kg, s.c.] and chronic vehicle injections. Values represent the mean ± S.E.M. number of episodes of the indicated behavior counted for 30 min post-antagonist injection; see Methods for details).* *$p<0.05$, **$p<0.01$, *Mann-Whitney U test vs. vehicle controls (n = 9 for U50; n = 5 for vehicle).*

	TREATMENT			
ENDPOINT	Chronic U-50,488H		Chronic Vehicle	
Yawning	**8.1**	**± 1.7 ****	**0.2**	**± 0.2**
Writhing	**11.0**	**± 5.8 ****	**0.0**	**± 0.0**
Rearing	**8.2**	**± 2.2**	**3.8**	**± 1.9**
Coughing	**2.2**	**± 1.4**	**0.0**	**± 0.0**
"Wet dog" shakes	**2.7**	**± 0.9 ***	**0.2**	**± 0.2**
Grooming	**1.8**	**± 0.5**	**0.8**	**± 0.2**
Chewing	**2.1**	**± 1.2**	**0.6**	**± 0.4**
Sniffing of bedding	**1.8**	**± 0.5**	**3.4**	**± 2.1**

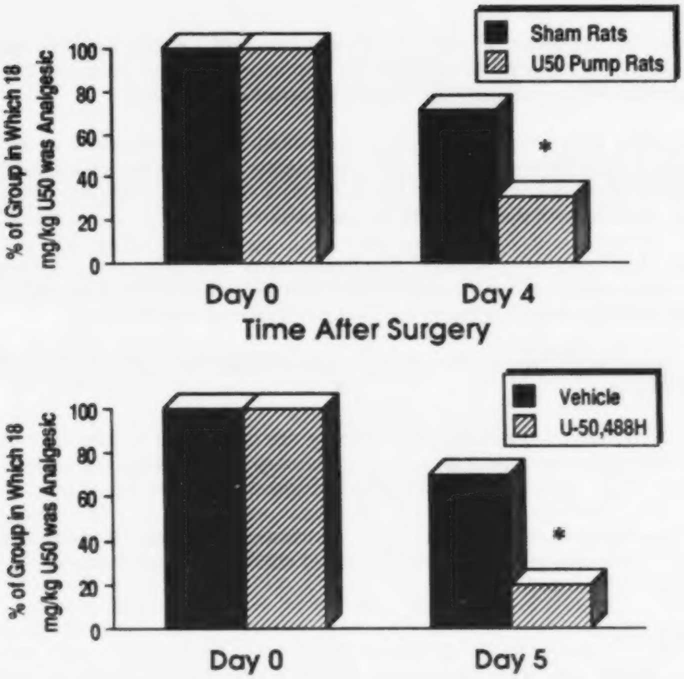

FIGURE 1. *Results of tolerance experiments. Upper panel: Study in which U50 was delivered via osmotic minipump (n=16 for U50; n=14 for sham operated). Lower panel: Study in which U50 was given by repeated injections (n=10 per group). Analgesic test was i.p. hypertonic saline writhing. *p<0.05 Fisher's exact test vs. control group. See Methods for details.*

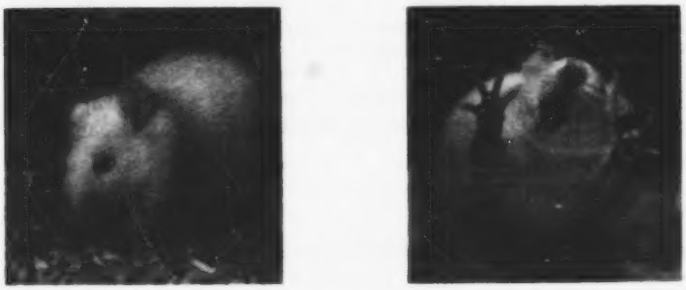

FIGURE 2. *Examples of rats exhibiting oral stereotypies resulting from injection of buprenorphine after chronic treatment with a* kappa *agonist.* *Left panel:* *constant gnawing of bedding/feces;* *right panel:* *persistent licking of cage walls.*

SUMMARY

1. When U50 was given to rats over 5 d by twice-daily s.c. injection (but not when delivered by osmotic minipump), buprenorphine and naloxone each precipitated strong, qualitatively distinct, behavioral syndromes.

2. The same dose of buprenorphine provoked similar behaviors in rats given chronic U50 and chronic TIF (analogous s.c. injection protocols), suggestive of neuroadaptation to *kappa* agonists as a class. This adaptation clearly contrasts with that to chronic *mu* agonists.

3. The buprenorphine-induced syndrome was characterized by oral stereotypies which had an onset of about 5 min and a duration greater than 4 hr. The intensity was dependent on the dose of agonist injected.

4. The naloxone-induced syndrome was characterized by repetitive yawning and writhing.

5. If oral stereotypy, yawning and writhing are considered to represent an abstinence syndrome, then it will be necessary to use multiple or more selective *kappa* antagonists to fully unveil *kappa* dependence in the rat.

6. The present data indicate a strong trend toward the parallel development of tolerance in rats given a similar course of chronic U50 injections as those tested for physical dependence.

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Precipitation of Spinally Mediated Withdrawal Signs by Intrathecal Administration of Naloxone and the Mu-Receptor Antagonist CTP in Morphine-Dependent Mice

J. Shook, W. Kazmierski, V. Hruby and T. Burks

ABSTRACT

We evaluated the ability of naloxone and the mu receptor antagonist CTP (D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2) to precipitate withdrawal in morphine-dependent mice after intrathecal (i.t.) administration. The withdrawal syndromes elicited by naloxone and CTP given i.t. were compared to those of CTP or naloxone injected intracerebroventricularly (i.c.v.). When given i.t. or i.c.v., naloxone produced the classical syndrome of events including jumping, wet dog shakes, urination, defecation followed by diarrhea, and weight loss. There was no significant difference in the potency or efficacy of naloxone when it was given i.t. or i.c.v. The profile of withdrawal effects produced by i.t. CTP resembled that caused by i.c.v. CTP; both were different from that of naloxone. The withdrawal signs seen following both i.t. or i.c.v. CTP included wet dog shakes and defecation. Mice treated with i.t. CTP lost significantly less body weight than those treated with i.c.v. CTP. In addition, i.t. and i.c.v. CTP did not stimulate jumping behaviors or diarrhea. In contrast, while i.c.v. CTP resulted in increased incidence of urination, CTP given i.t. did not. These finding indicate that naloxone given spinally acts on mu receptors to precipitate wet dog shaking and defecation, but acts on other non-mu opioid receptors (i.e. delta and/or kappa) to cause jumping, urination, diarrhea and weight loss. The differential effects of CTP given i.c.v. or i.t. suggest that supraspinal mu receptors are more involved in gastrointestinal and urinary bladder function during dependence/withdrawal than their spinal counterparts. This in turn implies that another non-mu, spinal opioid receptor (i.e. delta or kappa) is a more important regulator of visceral function in withdrawal than spinal mu receptors. Both spinal and supraspinal mu receptor mechanisms may be related to wet dog shake behaviors.

INTRODUCTION

We have previously reported that the cyclic octapeptide CTP (D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2) displays selective mu receptor antagonist activity in radioligand binding assays (Pelton et al., 1985; Gulya et al., 1986), in the guinea pig ileum and mouse vas deferens bioassays (Shook et al., 1987a) and in mouse analgesic and gastrointestinal transit time tests (Shook et al., 1987b). CTP injected i.c.v. has also been shown to produce a distinct profile of withdrawal effects in morphine-dependent mice, which is different from that of naloxone given i.c.v. (Shook et al., 1986). CTP given i.c.v. caused dose-related increases in wet dog shakes, urination, defecation and weight loss which were equal to, or greater in magnitude, than those caused by i.c.v. naloxone (Shook et al., 1986). CTP given i.c.v. did not stimulate withdrawal jumping or diarrhea, even at very high doses (Shook et al., 1986). The differences in withdrawal signs elicited by i.c.v. CTP and i.c.v. naloxone may be attributed to their differences in receptor selectivity. Naloxone, which interacts with all known opioid receptors, should precipitate withdrawal effects associated with all receptor types rendered dependent by chronic morphine treatment, whereas CTP should only precipitate withdrawal effects associated with the mu receptor. Accordingly, wet dog shakes, urination, defecation and weight loss are probably mediated by supraspinal mu receptors, precipitated by i.c.v. CTP while jumping and diarrhea may be mediated by other non-mu, supraspinal opioid receptors (ie. delta or kappa).

In the present study we evaluated the ability of spinally-administered naloxone and CTP to precipitate withdrawal in morphine-dependent mice. Naloxone and CTP given i.c.v. were included as standards of reference. All observations were limited to the first 15 min following injection ino order to avoid misinterpretation of data due to redistribution of drugs outside of the spinal compartment.

METHODS

One 75 mg morphine-base (or placebo) pellet was implanted subcutanously in male ICR mice (20-25g) under ether anesthesia. Following pellet implantation, mice were housed 3 per cage under standard 12 h light, 12 h dark conditions, and received food and water ad libitum. Experimentation started at 72 h following pellet implantation. Each mouse was weighed, and injected i.t. or i.c.v. with naloxone, CTP or distilled water (vehicle). Mice were then observed for the 15 min period immediately following injection. The behavioral and physiological signs recorded were jumping, wet dog shakes, urination, defecation and diarrhea. Body weights were redetermined at 15 min following injection, and loss of body weight was calculated as a percent of each animal's pre-injection body weight.

CTP was synthesized as previously described (Pelton et al., 1986). Naloxone was purchased from Sigma (St. Louis, Mo.). Morphine base pellets were received from the National Institute on Drug Abuse (Rockville, Md.).

Intrathecal (i.t.) injections were carried out according to the methods of Hylden and Wilcox (1980) in a total volume of 5 ul. Intracerebroventricular (i.c.v.) injections were made in a total volume of 3.0 or 3.3 ul, according to the methods of Porreca et al., (1984).

Statistical differences were determined by ANOVA and the t-test for grouped data as described by Tallarida and Murray (1981).

RESULTS

Morphine pelleted mice injected i.t. with distilled water showed mild weight loss (loss at 15 min was 1.6+.4%) and defecation (71%). Those injected with i.c.v. distilled water also showed weight loss (loss at 15 min was 1.6+.2%) and defecation (75%).

No jumping, wet dog shakes, urination or diarrhea were seen in placebo or morphine pelleted mice injected with distilled water given i.t. or i.c.v.

Naloxone given i.t. stimulated dose-related increases in jumping, wet dog shakes, urination, defecation and diarrhea (Table 1) and loss of body weight (Table 2). When injected i.c.v. at the same doses, naloxone produced a pattern of withdrawal responses identical to that produced by i.t. naloxone (Table 1). The magnitude of weight loss following i.t. naloxone was also similar to that caused by the same doses of naloxone given i.c.v. (Table 2).

CTP administered i.t. resulted in dose-related increases in wet dog shakes and the percent of mice to defecate, but did not stimulate jumping, nor did it increase the number of mice with diarrhea or micturition (Table 1). Weight loss was not significantly greater than control (i.t. distilled water) after i.t. CTP (Table 2). When given i.c.v., CTP produced the same profile of signs as equal doses of i.t. CTP, with the exceptions of (1) i.c.v. CTP increased the number of mice which urinated, and i.t. CTP did not (Table 1), and (2) i.c.v. CTP resulted in a significant degree of weight loss and i.t. CTP did not (Table 2).

For both naloxone and CTP, as the dose was increased, the time of onset decreased, with the 10 ug doses producing almost immediate effects. The 10 ug dose of CTP given subcutaneously (s.c) did not precipitate any withdrawal signs. The same dose of naloxone given s.c. did precipitate measureable withdrawal signs, but with delayed onset of action (greater than 10 min).

DISCUSSION

These results demonstrate that spinally located opioid receptors are affected by chronic morphine exposure, and contribute, at least in part to the withdrawal syndrome evoked by administration of an opioid antagonist. The similar response of morphine-

Table 1. Comparison of the effects of intracerebroventricular and intrathecal administration of naloxone or CTP in morphine-dependent mice.

		% TO DISPLAY WITHDRAWAL SIGNS			
		INTRATHECAL[a]		INTRACEREBROVENTRICULAR[b]	
SIGN	DOSE	NALOXONE	CTP	NALOXONE	CTP
WET DOG SHAKES	0.1	0	67	13	100
	1.0	83	100	100	100
	10.0	100	100	100	100
JUMPING	0.1	0	0	0	0
	1.0	17	0	0	0
	10.0	100	0	100	0
URINATION	0.1	0	0	63	0
	1.0	100	0	63	33
	10.0	100	0	100	100
DEFECATION	0.1	67	50	38	50
	1.0	100	100	100	88
	10.0	100	100	100	100
DIARRHEA	0.1	0	0	0	0
	1.0	33	0	38	0
	10.0	100	0	100	13

[a] n=6/dose

[b] n=8/dose

Tabel 2. Comparison of the effects of intrathecal or intracerebroventricular administration of naloxone or CTP on weight loss of morphine-dependent mice at 15 minutes following injection.

	% LOSS OF BODY WEIGHT AT 15 MINUTES			
	INTRATHECAL[a]		INTRACEREBROVENTRICULAR[b]	
DOSE (ug)	NALOXONE	CTP	NALOXONE	CTP
0.1	1.7+.5	1.2+.1	1.2+.2	3.3+.3*
1.0	4.0+.7*	1.2+.3	4.2+.4*	4.3+.5*
10.0	6.0+1.3*	2.0+.4	5.5+.5*	7.8+.5*

[a] n=6/dose

[b] n=8/dose

*significant difference from control ($p>0.05$)

dependent mice to naloxone given i.c.v. or i.t., suggests that spinal opioid receptors are affected by chronic morphine to about the same degree as supraspinal opioid receptors. We cannot be absolutely sure that spinal opioid receptors are solely responsible for causing its effects because the highest dose (10 ug) of naloxone tested was also active upon systemic administration. The delayed onset of action following s.c. and immediate onset following i.t. administration indicate that spinal receptors are atleast partially involved in mediating the effects of 10 ug i.t. naloxone, as it is unlikely naloxone could redistribute outside the spinal compartment in significant amounts immediately following injection.

The differences in withdrawal signs produced by naloxone and CTP are probably related to their differences in receptor selectivities. Naloxone interacts with all known opioid receptors, and CTP interacts only with the mu receptors. That naloxone (i.c.v. or i.t.) produced an array of effects consistent with, but greater than that caused by the mu-receptor antagonist CTP, suggests that chronic morphine exposure results in changes in other opioid receptors, in addition to the mu receptor. Based on the assumption that CTP will precipitate only those signs associated with the mu receptor, it appears that supraspinal mu receptors mediate wet dog shaking behaviors, gastrointestinal motility (but not secretion) and urinary bladder motility in morphine-dependent mice. The loss of urine and fecal weight may be responsible for the weight loss seen after i.c.v. administration of CTP. In contrast, spinal mu receptors do not regulate urinary bladder motility in morphine-dependent mice, but do influence gastrointestinal motility and wet dog shaking behaviors. These findings imply that other non-mu, supraspinal opioid receptors mediate jumping behaviors, gastrointestinal secretion, jumping, and urinary bladder motility. Although the identity of the particular non-mu opioid receptors that mediate these effects is not known, they may be delta or kappa related functions. Interestingly, supraspinal delta receptors have been proposed to exert intestinal antisecretory actions (Shook et al., 1987c), and spinal delta receptors have been shown to have an inhibitory influence on urinary bladder motility (Dray et al., 1984). Through combination of the effects produced by acute administration of highly selective receptor antagonists with the effects caused by receptor selective antagonists in morphine-dependent mice, we hope to identify further the fucntions associated with each receptor.

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Effects of 5,7-Dihydroxytryptamine Lesions of the Nucleus Accumbens in Rats Responding on a Concurrent Schedule of Food, Water and Intravenous Morphine Self-Administration

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ABSTRACT

The role of serotonergic innervations of the nucleus accumbens in the processes maintaining intravenous self-administration was assessed in rats responding on a concurrent schedule of food, water and morphine presentations. Five rats were trained on a concurrent fixed-ratio schedule of food and water presentation. They were then implanted with intravenous jugular catheters and bilateral injection guide cannulae into the central medial nucleus accumbens, made physically dependent on morphine and allowed to choose between intravenous morphine, food and water deliveries. A three-lever choice procedure provided almost continuous access to the three reinforcers. Dose-effect curves were determined by the substitution of the daily dose of morphine (3.3 mg/injection) with other doses (0.83-13.2 mg/injection) or eliminating drug injections (extinction) for 24 hour periods. The behavioral effects of 24 hour food extinction probes were also determined. The rats subsequently received bilateral microinjections of either the vehicle or 5,7-dihydroxytryptamine (5,7-DHT) into the nucleus accumbens. Following the lesion, response independent infusions of morphine were delivered for 24 hours at the previous rate of self-injection. The animals were placed back on the concurrent schedule and morphine dose-effect curves were redetermined. The 5,7-DHT lesion resulted in a significant dose-related decrease in morphine self-administration, and little or no effect on responding maintained by food or water presentations. Serotonergic innervations of the nucleus accumbens appear to participate in the neuronal activity mediating intravenous morphine self-administration.

INTRODUCTION

Innervations and projections of the nucleus accumbens are involved in the neuronal activity modulating the control of behavior by drugs of abuse. The role of these systems have been assessed using standard drug self-administration procedures. Lesions of dopaminergic inputs to the nucleus accumbens decrease intravenous cocaine (Roberts et al., 1977; Roberts et al., 1980), amphetamine (Lyness et al., 1979) and increase morphine (Smith et al., 1985) self-administration. Lesions of intrinsic neurons and afferents of the nucleus accumbens decrease cocaine and heroin self-administration (Zito et al., 1985). 5,7-Dihydroxytryptamine lesions of serotonergic innervations of this brain region increase both amphetamine (Lyness et al., 1980) and morphine self-administration (Smith et al., 1987).

Evaluations of the behavioral effects of neurobiologic manipulations require the use of multiple baselines to assess the specificity of the observed changes (Dworkin and Smith, 1987). For example, 6-hydroxydopamine lesions of the nucleus accumbens decreases the self-administration of cocaine but have little or no effect on heroin self-administration in rats trained to self-administer both drugs (Pettit et al., 1984). The behavioral specificity of two neurotoxin lesions of the nucleus accumbens has been determined using a procedure that allows for the simultaneous assessment of the reinforcing effects of food, water and morphine. 6-OHDA lesions have little or no effect on responding maintained by a concurrent schedule of food, water and intravenous morphine availability (Dworkin et al., 1987b), while kainic acid lesions result in a selective decrease in morphine self-administration (Dworkin et al., 1987a). This study evaluated the effects of 5,7-DHT lesions on responding maintained by the concurrent schedule of food, water and intravenous morphine.

METHODS

Five adult male inbred rats developed from the Fischer 344 strain, weighing between 275-325 g at the beginning of training were used. Each rat was implanted with a chronic indwelling jugular catheter and bilateral guide cannulae aimed into the central medial nucleus accumbens. The rats were continuously housed in standard operant conditioning chambers containing three retractable levers, a stimulus light above each lever, a tone source, a pellet dispenser, a water dipper and a motor driven syringe pump. An additional light mounted above the plexiglas top of the chamber illuminated the chamber from 5:00 p.m. to 5:00 a.m., providing a reversed 12 hr light/dark cycle. The training procedure used has been described in greater detail (Dworkin et al., 1984). Briefly, the subjects were trained to respond on three levers under a concurrent chained schedule of reinforcement. The first response on any lever resulted in the retraction of the other two. Nine additional responses on the extended lever resulted in the presentation of the chosen reinforcer. The rats were given 100 seconds to complete the nine responses after the initial response on one of the three levers (limited hold or LH). In addition, a 30 second time out followed the presentation of each reinforcer or elapse of the limited hold. The subjects had continuous 24 hour exposure to the schedule contingencies. During the food extinction probes food pellets were not delivered, the 3.3 mg/infusion dose of morphine was available and all other aspects of the schedule were unchanged.

Morphine sulfate was dissolved in a bacteriostatic 0.9% sodium chloride solution. Dose-effect relationships were determined, before and after lesions of the nucleus accumbens, by replacing the daily dose of morphine available (3.3 mg/injection) with other doses (0.83-13.2 mg/infusion) or eliminating drug injections for 24 hours. At least two determinations for each dose were made.

The rats were pretreated with desmethylimipramine (30 mg/kg, i/p/) 30 min prior to being anesthetized with methohexital (1 mg/kg, i.v.). Forty-five minutes after the desmetnylimipramine treatment, 0.5 ul of either the vehicle (isotonic saline-0.02% ascorbic acid) or 5,7-DHT (6 ug in isotonic saline-0.02% ascorbic acid) was bilaterally injected into the central medial nucleus accumbens over 7.5 min. The injection was made through 30-gauge injection cannulae extending 0.5 mm below the guide cannulae. They were left in place an additional 10 min after the neurotoxin injection.

RESULTS

Responding maintained by the schedule contingency was very similar to those previously reported (Dworkin et al. 1984). Increasing the dose of morphine available for self-administration resulted in a dose-related decrease in responding on the morphine lever and a modest dose-related increase in responding on the water lever. Responding on the food lever was not altered by changes in the morphine dose. The major effect of food extinction was a large increase in responding on the drug lever, while the most significant effect of morphine extinction was to almost completely eliminate responding on the water lever.

The effects of the vehicle lesion are displayed in Figure 1. The vehicle lesion of the nucleus accumbens did not result in any significant changes in the number of ratios completed on the levers that resulted in food or drug delivery. The only significant effect observed was a small increase in responding on the water lever when the largest dose of morphine (13.2 mg/infusion) was available and a greater decrease in responding on the water lever during morphine extinction sessions.

Figure 2 shows the effects of the 5,7-DHT lesion on responding maintained by food, water and morphine. The neurotoxin microninjection resulted in statistically significant decreases in the self-administration of the two lowest doses of morphine investigated.

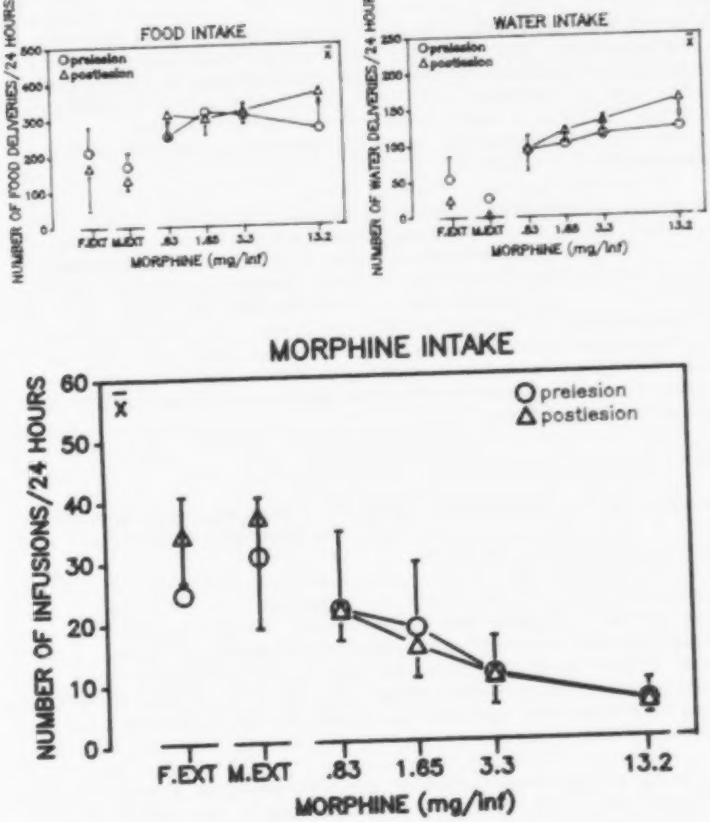

FIGURE 1. Dose-response curve for the number of fixed ratios completed on the food (top, left panel), water (top, right panel) and morphine lever (bottom panel). Symbols above "F.EXT" and "M.EXT" indicate the effects of food and morphine extinction probes, respectively. Points indicated by open circles represent the values observed before the vehicle lesion and the open triangles depict data collected following the vehicle lesion. The points are means of at least two determinations in two subjects. The vertical lines above the circles indicate +1 standard deviation and the vertical lines below the triangles indicate -1 standard deviation.

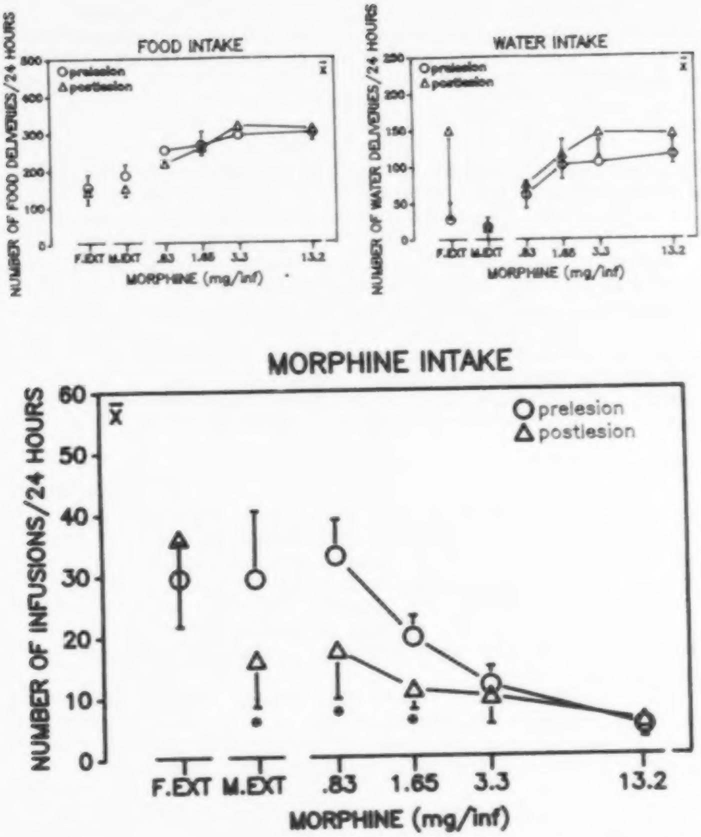

FIGURE 2. Dose-response curve for the number of fixed ratios completed on the food (top, left panel), water (top, right panel) and morphine lever (bottom panel). Symbols above "F.EXT" and "M.EXT" indicate the effects of food and morphine extinction probes, respectively. Points indicated by open circles represent the values observed before the 5,7-DHT lesion and the open triangles depict data collected following the neurotoxin lesion. The points are means of at least two determinations in three subjects. The vertical lines above the circles indicate +1 standard deviation and the vertical lines below the triangles indicate -1 standard deviation. The neurotoxin lesion did not result in any significant changes in responding on the food or water levers during any of the conditions investigated. The "*" indicate points that are statistically different ($p < .05$) for responding on the morphine lever.

Responding maintained by the largest dose of morphine however, was not altered by the lesion. Furthermore, the neurotoxin lesion did not result in any significant changes in responding on the food or water levers during any of the conditions investigated.

CONCLUSION

Serotonergic innervations of the nucleus accumbens are involved in the reinforcing aspects of morphine. Lesions of these neurons with 5,7-DHT decreased morphine self-administration. The observation that larger doses of the drug can overcome the effect suggests that the reinforcing efficacy of lower doses has been reduced. The effect on drug intake is not the result of non-specific changes in motor output since responding on the food lever was not altered and responding on the water lever was slightly increased. Thus, the decrease in responding occurred only on the morphine lever. Lesions of the serotonergic innervations of the nucleus accumbens selectively attenuated the reinforcing efficacy of morphine.

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An Approach to the Paradoxes of Experimental Opiate Dependence

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INTRODUCTION

Experimental analysis of early opiate dependence in the neuronal plexus of the guinea pig ileum has led to a formal theory on the nature of this type of dependence (Villarreal, et al., 1985, 1986, and 1987a). The new theory provides a formal definition of dependence which is analytical and quantitative. In the present paper we take up some of the outstanding paradoxes of experimental opiate dependence as these occur in different organisms and preparations with the purpose of testing whether or not the new theory throws light on these pharmacologic phenomena for which no reasonable solutions were previously known. It will be seen that these paradoxes of dependence can now begin to be understood as peculiar but nevertheless quite natural expressions of the basic mechanisms of abstinence and dependence proposed in the new theory, as parts of a single coherent whole. Space limitations allow only a succint presentation of the paradoxical phenomena and the reader is referred to the paper of Villarreal and Castro (1979) for details.

THE NATURE OF OPIATE DEPENDENCE

The theory of opiate dependence derived from work on the isolated ileum is based on the experimental features of the kinetic behavior of the abstinence response. There is clear evidence that opiate abstinence is mediated by a rate-coupled receptor system. There is also clear evidence that opiate dependence is a concretely definable hypertrophy of the rate-coupled receptor system which mediates abstinence. A rate-coupled system is operationally defined as a system mediating responses that are influenced by temporally-determined variables such as the rate constants of the drug-receptor-effector system as well as the rate of drug administration or of its disappearance from the medium. Responding is of course influenced by the simple passage of time inasmuch as rate-coupled systems give responses which show fade from an early peak effect. Basically, rate-coupling is taken to mean that the relationship between receptor occupation and the production of the primary pharmacologic stimulus is not fixed but changing, and that such relationship depends on temporal variables. We learned this idea from Pardo and Magaña (1959).

Before the development of the new model there was no mathematical frame of reference of drug-receptor theory to help us handle the rate-coupled nature of the abstinence response and even less the dynamics of modes of responding which gradually shift from rate-coupled towards occupation-coupled behavior. Diagram 1 of figure 1 presents the model system proposed to mediate the abstinence response precipitated by antagonists. It consists of receptor-effector elements which are activated by the onset of binding of antagonist molecules. After the onset of activation, the drug-receptor-effector complex becomes inactivated at a rate determined by its own rate constant of inactivation (kt). Such rate of inactivation is independent of the rate of chemical association (k1) or dissociation (k2) between drug and receptor. The inactivated effector may remain so for a while in a state of refractoriness and regain susceptibility for a new activation with a rate constant k4.

A second component of the theory proposes that opiate dependence consists in a gradual progressive increase in the half-life of the active states of the receptor-effector elements which mediate abstinence. This proposition is supported by experimental evidence and by studies of mathematical simulation of the behavior of the rate-coupled receptor model (Villarreal *et al.*, 1985 and 1986). The increased halflife of effector activation is represented mathematically by a reduced kt.

THE NATURE OF OPIATE ABSTINENCE PRECIPITATED BY ANTAGONISTS.

There are two aspects of the response of antagonist-precipitated abstinence which specially have to be considered. One is its intensity. The other is the quality of drug action of the opiate antagonist, namely, whether its action is abstinence-producing or abstinence-blocking or a mixture of the two. The important determinants of these aspects of drug action are the rate constants of the drug-receptor-effector system and the rate of access of the antagonist to the receptor pool.

To illustrate the model with a plausible physiological equivalent, an abstinence syndrome might be conceived as the macroscopic result of the temporal summation of excitatory depolarizing microevents, each produced by the onset of binding of an antagonist molecule to a receptor. The microevents take place in opiate-sensitive neurons and come to produce propagated neuronal action potentials when the microevents occur at a rate sufficiently high to allow them to summate and therefore depolarize the local membrane potential up to the neuronal threshold. Early in dependence, the rate of microevent production required to produce fully propagated responses is very high because the microevents are brief. Otherwise, if the rate of excitatory microevent production is very slow, as with a very slow infusion of an opiate antagonist, the microevents will occur but they will do so only at relatively long intervals. Under these conditions, the microevents will not summate

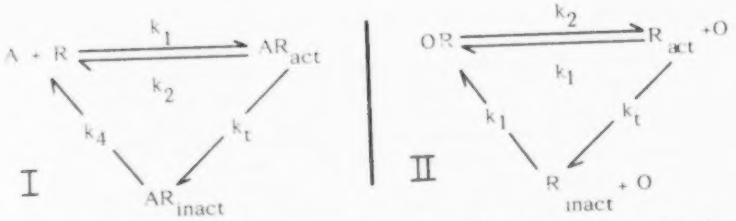

FIGURE 1. Two different kinetic mechanisms for opiate abstinence. Antagonist-precipitated abstinence is proposed to operate as in I: antagonist molecules can be free as "A" or bound to a receptor as "AR". Abstinence produced by opiate withdrawal is proposed to operate as in II: opiate molecules can be free as "O" or bound to a receptor as "OR". The condition of pharmacologic activation of the receptor-effector element is indicated by Ract. The constant kt expresses the spontaneous rate of decay of this active state. The abstinence response is proportional to Ract. Dependence is denoted by a reduced kt.

and consequently no propagated responses will be produced. Nevertheless, the receptors will be chemically occupied by the antagonist given in the slow infusion and thus the effects of a subsequent abrupt administration of the same or any other antagonist acting on the same receptor will be blocked. In contrast, in well developed dependence the microevents are long-lasting and so the rate of excitatory microevent production may be slower and still produce macroscopic abstinence because the microevents can easily summate and reach firing threshold levels even if they occur at relatively long intervals.

THE NATURE OF OPIATE ABSTINENCE PRODUCED BY OPIATE WITHDRAWAL.

Of course, an abstinence syndrome can also be produced by the simple withdrawal of an opiate which has been previously administered in a chronic manner. We now propose that this type of syndrome results from the operation of a mechanism analogous to that of antagonist-precipitated abstinence. However, the evidence suggests that in the abstinence responses produced by opiate withdrawal, the excitatory microevent must originate at the moment of termination of the bond between opiate molecules and their receptors (see II of Fig. I). The underlying process of dependence, i.e., the hypertrophy of the abstinence receptor system, denoted by a reduced kt, is in identical condition for the two different types of procedure that cause abstinence syndromes. Yet, the kinetic mechanisms of action of the two procedures are very different and, therefore, the abstinence responses obtained will be different. In the case of abstinence produced by opiate withdrawal, the k2 of the opiate and its rate of disappearance from the medium assume the same roles that in antagonist-precipitated abstinence are played by the k1 of the antagonist and by its rate of access to the receptor pool. It must be noted that in opiate dependence free receptors are not pharmacologically inert. The Ract elements have effects of opposite sign to the neurodepressant actions of the OR elements and thus the Ract constitute a strong mechanism for tolerance.

THE SUBLIMINAL ABSTINENCE SYNDROME

The mechanism of opiate abstinence must include a threshold step, which is likely to be the neuronal threshold for fully propagated neuronal action potentials. Further on we will refer to clear cut instances of subliminal abstinence conditions which appear to confirm this idea. Subliminal abstinence should be identified by the production of abstinence-like responses to the presentation of excitatory non-opiate non-antagonist stimuli applied at a time when the pharmacologic condition of the organism is bordering on abstinence. The response will be abstinence-like because only in opiate-dependent neurons with subliminal abstinence is there a coincidence of the two kinds of event: the subliminal narrowing of the distance to firing threshold and the additional effect of the non-opiate generalized excitatory stimulus.

PARADOXES OF EXPERIMENTAL OPIATE DEPENDENCE

1. Instances of Dissociation Between the Degrees of Dependence "Unmasked" by: a) Withdrawal of Opiate and b) Administration of an Opiate Antagonist.

1.1.- Dissociation of the intensities of abstinence obtained with the above two procedures in early dependence.

This dissociation was initially observed when the first major antagonist introduced, nalorphine, was first tested in opiate-dependent organisms. Previously, when investigators used to evaluate experimentally-produced opiate dependence by the intensity of the abstinence syndrome caused by withdrawal of opiate, they were used to the fact that it normally takes 3 to 4 weeks of opiate administration to be able to see a reasonably robust abstinence response to the withdrawal of the dependence-producing agent. Nalorphine was found to precipitate abstinence responses of important severity after only a few days of opiate treatment or even after infusions of morphine lasting only a few hours (Martin and Eades, 1961). There was debate and controversy over the issues raised by these findings. Of course, now that we count with the well validated fact of early dependence, the old controversies do not appear as strong. However, a formal account of the mechanism of the discrepancies observed between the abstinence responses obtained through the two procedures has not been advanced before. The reason for the differences is likely to be the different kinetic mechanism of action of the two procedures. Because the rates of dissociation of opiate agonists from their receptors are in general very much slower than the rates of association of opiate antagonists, the rates of production of excitatory microevents will be far slower for opiate withdrawal than for antagonist administration. Hence, the duration of the abstinence excitatory microevents must be much longer for an abstinence response to be obtained with simple opiate withdrawal than with the administration of an antagonist. Therefore, the theory predicts that abstinence syndromes will be produced by opiate antagonists much earlier in the process of dependence than such syndromes will be produced by opiate withdrawal.

1.2.- Dissociation of the intensities of abstinence obtained with the above two procedures in organisms treated with opiate analogs which produce low levels of dependence.

This point is quite important for the assessment of the dependence potential of new drugs. The chronic treatment with drugs of low dependence liability, such as many of the agonist-antagonist analgesics, leads to pharmacologic conditions where termination of drug treatment cause only abstinence syndromes of low intensity. However, if during the period of chronic treatment with one of these analgesics a high dose of a fast-acting opiate antagonists is administered, then more intense abstinence responses are obtained. One might ask which of the two forms of assessing dependence potential should be regarded as valid. Besides the practical issues here, the scientific dilemma posed by the question vanishes if one applies to the present problem the same considerations of section 1.1. above.

2.- The Intensity of Abstinence has an Upper Limit of Severity and Yet the Supersensitivity to the Abstinence-Precipitating Actions of Antagonists Continues to Grow Even After Such Upper Limit of Severity has been Reached.

The riddle associated with this paradox disappears with the notions of dependence discussed in this paper. The maximum level of abstinence severity is determined by the full activation of the entire set of opiate-sensitive neurons which participate in abstinence. The sensitization to the abstinence-precipitating actions of antagonists is determined by the increases in duration of the active states of the receptor-effector elements. Therefore, the sensitization to antagonists may very well continue to grow much beyond the point where maximum levels of abstinence severity can be just obtained. In turn, the limit of sensitization to antagonists is given by the law of hyperbolas (Villarreal, et al., 1987a).

3.- The Paradox of Control Baselines with Non-Zero Dependence.

Many reports in the literature contain instances where animals or experimental preparations never previously treated with opiates respond with abstinence-like phenomena to the administration of large doses of opiate antagonists. The new theory also handles this problem in a clean fashion. The abstinence effector system is there to start with, but in a state of varying degrees of atrophy. The theory predicts that preparations with effectors in an incompletely atrophied state will give abstinence responses to very large doses of antagonists administered at high rates. It must be noted that the non-zero dependence baselines are commonly obtained in vitro where high doses of antagonists administered at high rates are easily given.

4.- The Paradox of Abstinence Responses Precipitated by Antagonists

Weeks or Months After the Last Administration of a Dependence-Producing Opiate.

Abstinence-like syndromes can be produced in monkeys or rodents by the administration of opiate antagonists when these agents are given weeks or months after termination of a chronic period of opiate treatment. At the time of the paradoxical precipitation of abstinence most if not all of the opiate administered long before should have been eliminated from the organism. Then, if the opiate is gone, how is it that an antagonist produces abstinence. These organisms would be in abstinence for as long as dependence was present if abstinence was simply mediated by an occupation-coupled receptor mechanism. It is more reasonable to consider all the evidence in favor of the idea that abstinence is mediated by a rate-coupled receptor system and that dependence is a hypertrophy of such system. The hypertrophy caused by the dependence-producing opiate of the present paradox might then decay at some slow rate of its own after termination of opiate. High rates of microevent production can be achieved with high doses of antagonists administered rapidly when the effector hypertrophy produced by dependence has decayed perhaps a great deal but has not disappeared altogether.

5.- Paradoxes About the Abstinence-Precipitating Actions of Opiate Antagonists.

On of the most interesting features of these pharmacologic actions is that specific opiate antagonists differ in their intrinsic activities for the precipitation of abstinence. There is even evidence, for certain antagonists, of an orderly variation of intrinsic activity within each compound when these agents are tested at different levels of opiate dependence. Such paradoxes of opiate antagonists are impossible to explain in terms of simple occupation drug-receptor theory. The rate-coupled model accounts in an uncomplicated fashion for the paradoxes concerning the intrinsic activity of the abstinence-precipitating antagonists (Villarreal, *et al.*, 1987b).

6.- The Subliminal Abstinence-Syndrome.

Villarreal and Castro (1979) review striking cases of abstinence-like responses obtained in opiate-dependent organisms through the application of excitatory experimental interventions unrelated to the removal of opiate from its receptor site of action. Collier (1979) and his coworkers have drawn attention to what they have designated the quasi-abstinence syndrome whereby certain non-opiate excitatory drugs enhance the abstinence-precipitating effects of opiate antagonists. The group of phenomena of quasi-abstinence could perhaps be regarded as a part of the set of effects of the subliminal abstinence syndrome.

Finally, there is another set of interactions, discussed by Villarreal (1981), which can be added to the set of conditions where subliminal abstinence operates. In the presence of mild dependence, strong abstinence responses can be obtained with antagonists if any of the following is applied: ouabain, low extracellular calcium, electrical stimulation. These interventions are directly membrane depolarizing except low calcium which, nevertheless, augments the self-regenerating effects of membrane depolarization.

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Development of A Computerized System for Inventory of Controlled Drug Substances

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ABSTRACT

An automated drug inventory system was developed to facilitate record keeping requirements for controlled drug substances. A hand-held barcode scanner and a computer-accessible top-loading electronic balance are used to identify and weigh samples each time they are removed from or returned to the safe. A computer compares the initial sample weight to its last recorded weight to assure that no discrepancies occurred prior to sample use. Upon return, the computer compares the difference in initial and return tare weights to the stated amount of drug used to assure accuracy of the written inventory record. Unreconciled errors are immediately brought to the attention of the safe custodian. During its first year of operation, 2791 sample out/sample return transactions on 381 drug samples were tracked by the system. The most common errors detected by the system include sample loss of moisture (9), sample absorption of moisture (4), wrong sample used (4), incorrect weight recorded (4), and sample used without log entry (4). Importantly, all errors were immediately identified and reconciled. At the end of each working day, a printout of all daily transactions shows sample use, possible errors, and whether all samples have been returned to the safe. A printout of the total drug inventory is immediately available when called for. This system provides enhanced vigilance and security, and has been readily accepted by all investigators using controlled drug substances in our laboratory.

INTRODUCTION

Drug Enforcement Agency (DEA) regulations require that investigators maintain complete and accurate records for the receipt and dispensing of controlled drug substances. In practice, many investigators keep drug samples in a locked drawer or safe and record drug usage on hand-written inventory sheets. We have found that, even in the best laboratories, inadvertent inventory errors arise when an investigator (a) forgets to record the use of a sample, (b) uses the wrong sample, (c) records the sample usage on the wrong inventory page, (d) records incorrect data due to a misplaced decimal point or an incorrect balance reading, or (e) makes addition/subtraction errors in the log book. This can lead to serious and time-consuming problems when reconciling inventory records prior to the annual dump-weigh drug audit. We have now developed an automated inventory system to guard against these errors. This procedure supplements current DEA-approved inventory methods.

METHODS

Our system was implemented by connecting a computer-accessible top loading balance (Mettler PE 1600) to our existing laboratory computer system. A hand-held barcode scanner (DEC RT701) was connected in-line with the computer terminal and individual barcode labels were affixed to each of the 300+ samples in our safe. A custom-written Fortran program was developed to operate the system functions and provide a user-friendly step-through guide for inventory transactions. In practice, each time a sample is used, a technician enters his/her initials on the computer keyboard, then uses the barcode scanner to identify the sample. After the sample is placed on the balance, the computer compares the sample tare weight to the last recorded reading to assure that no discrepancy has occurred prior to sample use. The procedure is repeated when the drug is returned to the safe, with the exception that the computer then compares the difference of initial/return tare weights to the stated amount of drug used (entered on the keyboard). If the numbers agree within a programmed accuracy level (± 0.02 gm), the technician completes the entry by adding the experiment name and notebook page number. The computer indicates an error condition whenever there is a discrepancy in tare-weight readings. Errors that remain unreconciled after two additional weighing opportunities are flagged and brought to the immediate attention of the safe custodian. The sample is not released for further use until the source of the error can be identified and corrected. Each attempted inventory access is recorded and printed at the end of the day. All data are recorded in a permanent computer file for further reference and database applications.

RESULTS AND DISCUSSION

During the first year of operation, 2791 sample out/sample return transactions were recorded on the automated drug inventory system. Thirty-seven technicians and investigators are authorized to use drug samples from the controlled drug inventory; 4 of these are "safe custodians" who may unlock the narcotics safe. The system currently tracks 381 individual samples, of which 20 most frequently used samples account for 92% of all record entries.

Errors detected and corrected by the system include:

Loss of weight -- sample loss of moisture	9
Gain of weight -- sample absorption of moisture	4
Wrong sample used	4
Incorrect weight recorded	4
Sample used without log entry	4
Loss of weight -- powder stuck to weighing paper	1

Total = 26 errors

Significant benefits derived from use of the system include:

- Enhanced vigilance and security
- Fast, accurate, easy to use system
- Computer-read bar code on each sample assures sample accuracy
- Automated record keeping prevents addition/subtraction errors; assures record accuracy
- System indicates "who" has the sample if not in safe; simplifies "sample searching" time
- Printout of daily log entries indicates samples not returned to safe at end of day
- Permanent computer log permits rapid searching of sample data
- System is easily adapted to tracking both scheduled and non-scheduled drugs
- System readily accepted by technicians and investigators

During our annual audit, all dump-weighed C-I and C-II drugs had weights within specified accuracy levels. All 49 samples emptied since the audit had expected zero balance levels on the written drug inventory record; no unresolved inventory errors were found.

Twelve samples found to have slightly increased or decreased weights during storage were of particular interest. Seasonal variations in moisture content and/or slight volatilization of the drug substance were identified as the likely sources of error. Samples gaining 0.04 to 0.09 gm weight during the spring-summer period included morphine sulfate, morphine pellets, and phenobarbital sodium. Initial sample weights were between 15 and 82 grams. Samples loosing 0.03 to 0.07 gm weight during the fall-winter period included codeine phosphate, phenobarbital sodium, phencyclidine HCl, normeperidine HCl, amphetamine sulfate, and amphetamine HCl. A 14 gm sample of codeine base having an 0.50 gm loss of weight over a 5 month fall-winter period was verified by the manufacturer to have an unstable hydrate (0-7% water content); moisture analysis demonstrated that the sample decreased from 5.36% to 0.2% moisture content during the storage period. This loss of moisture more than accounts for the weight discrepancy. Hexobarbital sodium and chloral hydrate were found to be slightly volatile in their solid form; loss of weight could be prevented by tightly sealing the sample bottles with parafilm.

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Assessment of Opiate Tolerance and Dependence *In Vitro*

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ABSTRACT

Experiments were performed to induce opiate tolerance and physical dependence in vitro and assess the applicablility of the procedures for studying the basic mechanisms in tolerance and dependence development. It was established that tolerance and physical dependence could be demonstrated in the excised guinea pig ileum and mouse vas deferens by direct incubation with an opiate agonist. The selectivity of the response to opiates was determined by experiments demonstrating stereospecificity, naloxone reversibility and cross-tolerance. The magnitude of tolerance and physical dependence was found to be dose, time, and agonist type-dependent. A standardized degree of tolerance and dependence were induced by using a dose of agonist sufficient to inhibit electrically stimulated contractions by 50% (IC50). In the isolated guinea pig myenteric plexus of the longitudinal muscle, incubation of the IC50 of an agonist at 37°C for one hour produced a 2 to 5-fold tolerance as evidenced by the increase in its IC50. The rank order of decreasing potency in developing tolerance was morphine, normorphine and methadone. Physical dependence on the opiate was established by a contractual response to naloxone or a supersensitive response to electrical stimulus after removal of the agonist by washing. The degree of dependence developed was found to be proportional to that of tolerance. Similar incubation experiments on the mouse vas deferens with opioids also resulted in the development of tolerance and dependence. Although naloxone failed to evoke a contractual response, it enhanced dose-dependently the sensitivity of the preparation to electrical stimulus. Based on these observations, we conclude that our data are in agreement with ther hypothesis that opiate tolerance and physical dependence development are related processes.

INTRODUCTION

To elucidate the mechanisms involved in opiate tolerance and physical dependence studies have been generally performed on experimental animals receiving repeated or sustained administration of an agonist. However, due to the complexities of analyzing multiple pharmacologic responses in the whole animal, attempts have been made to develop in vitro models in which specific effects related to tolerance and dependence can be assessed more directly. The guinea pig ileum and mouse vas deferens are commonly used for studying acute opioid effects and more recently, these preparations were found to be suitable also for assessing opiate tolerance and dependence development. In the present communication, we will review some of the latter work and present additional data to demonstrate the applicability of the ilea and vas for assessment of opiate tolerance and dependence.

1. Guinea pig ileum

Assessment of tolerance: Three decades ago, it was established that opiates inhibit electrically induced contractions of the excised guinea pig ileum (Paton, 1957). Later, Kosterlitz et al., (1968) showed that the action of opiates on the myenteric plexus of the longitudinal muscle is dose-dependent, stereoselective and naloxone reversible. Moreover, the rank order in potency of the various opiate agonists to inhibit the ileum was found to be similar to that with respect to antinociceptive activity in animal models (Kosterlitz et al., 1968). Subsequently, attempts were made to adapt the guinea pig ileum preparation for tolerance studies.

The presence of tolerance in the myenteric plexus of the ileum excised from guinea pigs implanted with morphine pellets was described by Goldstein and Schulz (1973) and Schulz and Herz (1976). Tolerance was also produced in vitro in this preparation by incubating segments of the isolated ileum from naive animals with an agonist for 18-22 hours at 4°C (Hammond et al., 1976) and at 22°C (Opmeer et al., 1978, Collier et al., 1981).

Since tolerance in the ileum can be detected even after a single dose of morphine (Huidobro-Toro et al., 1978), we attempted to simplify the technique for inducing tolerance in this preparation. Accordingly, a systematic study was initiated using strips of longitudinal muscle attached to the myenteric plexus excised from naive guinea pigs. Segments of the ileum were incubated with an opiate agonist at 37°C at various concentrations and time intervals.

The results indicated that a high degree of tolerance can be induced under such conditions (Rezvani et al., 1983). Also, the development of tolerance and dependence in vitro was found to be agonist and time dependent. Morphine induced the highest degree of tolerance followed in order by normorphine and methadone. Incubation of the ileum with 2x IC50 of morphine induced a 22-fold tolerance. Under the same conditions, an 18 and 10 fold tolerance to normorphine and methadone was developed respectively (Rezvani et al., 1983).

The validity of this simplified *in vitro* procedure for producing tolerance was demonstrated by its compatibility with data obtained on intact animals with respect to stereospecificity, naloxone reversibility and cross-tolerance. Moreover, development of tolerance to morphine did not affect the responsitivity of the ileum to norepinephrine or acetylcholine. In addition, the tissues rendered tolerant to morphine also exhibited physical dependence as evidenced by a contractual response to naloxone. Finally, the utility of the procedure was further buttressed by the observation that segments of the ileum rendered tolerant to morphine, regained their sensitivity to morphine after pre-incubation with low concentrations of dynorphin 1-13 (Rezvani and Way 1984). These results are compatible with those obtained earlier by Tulunay *et al.*, (1981) who found that dynorphin enhances the antinociceptive effects of morphine in morphine-tolerant mice. Thus, the results clearly establish that *in vitro* induction of tolerance in the guinea pig ileum can be utilized for the assessment of opiate tolerance.

Assessment of dependence: Ehrenpreis *et al.*, (1972) demonstrated that guinea pig ileum strips incubated for 10 minutes with morphine exhibit a contractile response upon exposure to naloxone. This finding was confirmed by Schulz and Herz (1976), who also found that the intensity of the contraction was proportional to the degree of tolerance. Collier *et al.*, (1981) incubated segments of ileum with morphine for 24 hours at 4°C and observed that the response to naloxone was stereospecific and concentration dependent.

While developing the simple method of inducing opiate tolerance in the guinea pig ileum, we confirmed that exposure of the tissues to naloxone causes a contraction and that the magnitude of the contractile response could be correlated with the degree of tolerance developed to each agonist. We noted further that upon application of an electrical stimulus to the dependent tissue, a supersensitive response could be obtained, as evidenced by an increase in twitch height (Rezvani *et al* 1983). These observations prompted us to investigate whether the supersensitive response could be utilized for the quantitative assessment of physical dependence.

After the ileum from an untreated animal was excised and stimulated electrically for 15 minutes, the twitch height to a given stimulus was measured. Subsequently, the tissue was rendered tolerant to and physically dependent on morphine to varying degrees by incubating with varying concentrations of morphine for two hours at 37°C. With 1x, 2x or 5x IC50 a 7, 10 and 15 fold tolerance, respectively, was observed. The tolerant-dependent tissue was then washed thoroughly at five minute intervals for one hour with Ringer solution to remove the morphine, and its sensitivity to the initial electrical stimulus was redetermined. The ratio of the twitch height obtained after and before treatment provided an index of the degree of physical dependence.

Under the above conditions, a supersensitivity response to morphine, but not to ethylketocyclazocine (EKC), could be obtained. Furthermore, the effect was found to be stereospecific as evidenced by the demonstration of a supersensitive response to levorphanol but not to dextrophan after tolerance had developed. Both the development of supersensitivity and tolerance could be blocked by co-incubation of morphine with naloxone. A supersensitive effect was not elicitable with the kappa agonists, dynorphin and EKC. Met-enkephalin was effective but leu-enkephalin was not (Rezvani and Way 1983). The development of supersensitivity presumably involved protein synthesis, since it could be blocked with chloramphenicol as described previously *in vivo* (Della Bella and Fugeni 1978, Rezvani and Way 1983).

2. Mouse vas deferens

Assessment of tolerance: Henderson *et al.*, (1972) reported that opiates inhibit adrenergic neuroaffector transmission in the mouse excised vas deferens dose-dependently. Later, Schulz *et al.*, (1983) observed that the isolated vas deferens from mice treated chronically with morphine showed a high degree of tolerance. In a follow-up study, (Schulz and Wuster 1984) carried out cross-tolerance studies with a delta agonist DADLE (D-Ala-D-Leu enkephalin) and a mu agonist, sufentanil. The vas infused with DADLE showed about an 800 fold tolerance, but those treated similarly with sufentanil or EKC exhibited only a 5- and 2-fold increase, respectively. Recently, we attempted to circumvent pellet or mini pump implantation in the whole animal by inducing opiate tolerance dependence *in vitro*.

Mouse vas deferens were incubated with a mu or delta agonist *in vitro* for 2 hours at 37°C. After incubation of the tissues with 10^{-6} morphine, a 4-fold tolerance could be obtained. Under the same conditions, with 1xC50 of DADLE, a 6-fold tolerance developed. These studies are still in progress.

Assessment of dependence: Earlier experiments to demonstrate physical dependence in the mouse vas deferens were unsuccessful. In tissue tolerant to different opioid ligands, a contractile response to naloxone could not be elicited. A plausible explanation for the failure to elicit a naloxone contracture might be due to the fact that transection of the nerve somata during removal of the vas reduces its responsitivity (Schulz and Wuster 1984). However, North and Vitek (1980) were able to establish that physical dependence might occur in this tissue and that mouse vas deferens tolerant to normorphine display supersensitivity to electrical stimulation after exposure to naloxone. This phenomenon is analogous to the supersensitivity response of the morphine-tolerant guinea pig ileum to electrical stimulation upon exposure to naloxone. In order to investigate whether the supersensitivity to electrical stimulation after development of tolerance/dependence can be quantified in the mouse vas deferens, we performed the following experiment.

The vas deferens from a naive mouse was mounted in an organ bath and incubated with 1xIC50 of DADLE for one hour after which the tissue was subjected to electrical stimulation in the presence of increasing concentrations of naloxone. The amplitude of the twitch to a given electrical stimulus was found not only to increase from 29 to 41mm, but was also shown to be proportional to the concentration of naloxone added. Thus, these results indicate that dependence on opiate can be induced in the mouse vas deferens *in vitro* and the degree of dependence can be quantified by determining the antagonist ED50 for evoking such a phenomenon.

Based on the varying degrees of tolerance obtained with different ligands, the low degree of cross-tolerance exhibited between the ligands and the inability to detect dependence development with any of the ligands, upon exposure of the tissue to naloxone, Schulz and Wuster (1984), concluded that different processes were involved in tolerance and dependence development.

These conclusions can be criticized on at least two grounds. Firstly, since different responses were used to assess tolerance and physical dependence, it is unfair to equate the sensitivity of the measures for determining tolerance and physical dependence. Secondly, although the authors used the most selective ligands available for each receptor type, it should be noted that at high concentrations, the ligands also have affinity for other types of opioid receptors. For example, (EKC) has been shown to bind to the mu and delta as well as to kappa sites. Also, DADLE has been known to have considerable affinity for the mu receptor. Considering that in the experiment by Schulz and Wuster (1984), fairly high concentrations of the agonists were present in both the *in vivo* and *in vitro* studies, the activation of more than one receptor type cannot be excluded.

Our present experiments lead us to arrive at conclusions contrary to those of Schulz and Wuster (1984). Our observations establish that tolerance induced in the guinea pig ileum or the mouse vas deferens by direct incubation with an agonist is accompanied by the development of physical dependence. The fact that tolerant tissue exhibited a supersensitive response to electrical stimulation after removal of the agonist by washing, suggests that opiate tolerance and dependence are related since both tolerance and dependence were assessed by changes to electrical stimulation involving a common neural pathway.

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Chronic Opioid Treatment of Intractable, Non-Malignant Pain

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INTRODUCTION

Despite the merits of many new pain therapies the various treatments may be individually or even collectively insufficient to achieve adequate pain control in some chronic pain patients who have non-malignant conditions. Consequently, these patients require continuous administration of opioids. Little is known, however, about these patients since they have not been systematically studied. In 1979, due to demand in our geographic area, we began accepting patients on a referral basis who had severe, non-malignant pain and who were already dependent upon the daily administration of opioids for pain relief. Reported here are the 52 patients evaluated and treated by us in this pilot program between 1979 and 1986.

PROCEDURES AND METHODS

All patients were referred by a physician, hospital, or government agency. Requirements for acceptance by us were that the referral source and patient believed that chronic opioid administration was essential for adequate pain relief and that all other pain treatments acceptable to the patient had been unsuccessfully attempted. Pain was classified as either structural or non-structural. In the former case, there was documented evidence of permanent, irreversible destruction of a bodily structure such as bone, nerve, or muscle. Non-structural pain consisted of cases in which damage to a bodily structure could not be documented, such as headache or neuralgia.

In four cases of generalized pain of unknown cause, special diagnostic studies including immunologic tests and skin biopsies were done. A urine specimen was analyzed for 50 drugs by thin layer chromatography to help determine what drugs the patient was self-administering.[10] Patients were initially prescribed the opioids that they had previously been taking. Enough opioids were prescribed to maintain the patient between visits. Each patient gave written, informed consent for opioid maintenance and the consent clearly stated that opioid dependence would likely be for a life-time.

In an attempt to minimize the daily opioid dosage and enhance pain relief, every patient received multiple sequential trials of non-opioid analgesics and opioid potentiators including non-steroidal, anti-inflammatory agents, tricyclic antidepressents, neuroleptics, antihistamines, and amino acids, tryptophan, tyrosine, leucine, and phenylalanine. Various physiotherapies were concomitantly given to all patients with structural pain involving the spine, joints, and/or extremities. Intralesional corticoids were administered to all patients who demonstrated trigger points of pain. Ancillary medical conditions were treated by us in conjunction with the referring physician.

Pain control was assessed by self-report on each clinic visit and judged by the attending physician as adequate, partially adequate, or inadequate. When pain control was clinically judged anything but adequate, attempts were made to increase the opioid dosage, prescribe an ancillary pain medication or potentiator, incorporate a non-drug therapy, or switch opioid drugs, particularly to an opioid which has a longer duration of action than the one being taken. Plasma concentrations of the therapeutic opioid were determined in 11 patients who complained of inadequate pain relief. Blood sampling was done one to two hours after the patient was observed to administer their opioid. In patients who demonstrated no detectable opioid in their plasma, non-absorption from the gastrointestinal tract was assumed, and a different opioid was prescribed. Five patients experienced recurrent, acute exacerbations of pain and who required frequent hospitalizations or emergency room visits to obtain intramuscular injections of opioid were taught to utilize hydromorphone or morphine suppositories to prevent this need.

CLINICAL CHARACTERISTICS OF PATIENTS

Patients ranged in age from 20 to 77 years with a mean of 48.6 ± 14.6 S.D. They were about evenly divided between males and females. Only 11 (21.2%) were gainfully employed. Duration of time they had taken opioids for chronic pain prior to treatment by us ranged from .75 to 40 years (mean 12.0 ± 10.6 S.D.). The longest duration of 40 years was in a man who first began chronic opioid administration for a battle wound sustained in 1941 during World War II, and which resulted in a loss of a leg. Second longest duration was a 70 year old woman who began hydromorphone injections in 1947 for trigeminal neuralgia. In addition to the primary cause of pain, patients also had numerous other medical conditions and took various pharmacologic agents. Four patients used their opioids by injection because they either had a gastrectomy and/or previously found that oral opioids had no effect.

CAUSE OF PAIN

The etiology of pain in 48 (92.3%) patients was obvious and well-known to the patient and referral source. Four(4;7.7%) patients were referred with pain of unknown cause. Opioids had been prescribed due to intractable muscle, joint, and/or abdominal pain. Forty two of the 52 (80.8%) patients demonstrated structural abnormalities of tissue as their primary source of pain. The most common causes of pain were arthritis and post-trauma or postsurgical destruction of tissue (Table One). Trauma cases were caused by auto accidents, falls, or gunshot wounds that resulted in crushed vertebrae or destruction of other bony structures. Post-surgical pain was primarily the result of multiple back or limb operations or tissue destruction and/or adhesions resulting from abdominal surgery. Three (3;5.8%) patients had surgery for removal of cancers. Other causes of structural pain were varied and included some relatively uncommon medical conditions such as Gaucher's disease and porphyria. Ten (10;19.2%) patients had non-structural pain including seven (7;13.5%) with intractable headaches, two (2;3.8%) with trigeminal neuralgia, and one (1;1.9%) with Restless Legs Syndrome. Of the four patients with pain of unknown origin, two proved to have scleroderma, one had systemic lupus erythematosus, and the other had gout.

OPIOIDS USED FOR TREATMENT

Four techniques were primarily utilized to control pain. One was simply raising the dosage of the opioid which the patient was accustomed to taking. A second was changing the patient to a longer-acting opioid, usually methadone or oxycodone in place of codeine, meperidine, or hydromorphone. Some patients, however, complained that the longer acting opioids were not as effective in relieving pain as the short acting opioids. Other patients achieved adequate pain relief when low dosages of methadone, 10 to 40 mg per day, were given concomitantly with codeine or another short-acting opioid which likely suppressed intermittent withdrawal symptoms.[8] Five patients experienced episodic acute exacerbations of pain which were controlled by hydromorphone or morphine suppositories. Patients would supplement their regular daily dosage of opioid with the suppositories in a manner to eliminate repeated visits to an emergency room or hospital to obtain additional opioids during acute exacerbations of pain (Table Two).

Plasma concentrations of opioid were determined in 11 (21.2%) patients who complained of inadequate pain relief. Four patients who had plasma codeine determinations revealed two, who administered 600 and 960 mg per day respectively, with non-detectable levels while the other two who administered about 300 to 500 mg per day showed 322 and 1160 ng/ml. Five patients with methadone plasma determinations showed one, who administered 100 mg per day, with a non-detectable level while the other four consumed 40 to 160 mg per day showed concentrations ranging from 108 to 1470 ng/ml. Plasma concentration in one patient who administered 1000 mg of propoxyphene per day was 376 ng/ml and in one patient who administered about 80 mg per day of oxycodone was 600 ng/ml. Patients without detectable plasma levels of opioid were assumed to have inadequate absorption of the opioids and were switched to another oral or injectable opioid which, in all cases, adequately provided pain relief. Those patients who had detectable plasma levels were assumed to adequately absorb their opioid and were clinically managed by raising their daily dosage of opioid or adding other non-opioid therapies until adequate pain relief was achieved.

OUTCOME OF TREATMENT

After necessary and systematic changes in opioid and dosage were done as described above, 46 of the 52 (88.5%) patients were clinically judged by attending physicians to achieve adequate pain control on most days of the week according to the patient's self-report. Six (6;11.5%) were judged as partially controlled. Once pain control was adequate, the daily intake of opioids remained relatively constant and seldom varied over 10 to 15% each day according to reports by patient and family.

In the year prior to this report, the 32 patients still in treatment were hospitalized only four times and made eight emergency room visits. The most common complications observed were constipation (20;38.5%) and edema (12;23.1%) of the extremities. The latter would temporarily remit with administration of adrenal corticotropin (ACTH). One patient with systemic lupus erythematosus and gout who required 80 mg per day of oxycodone for pain relief developed adrenal insufficiency which required corticoid replacement.[11,12]

SUMMARY

There is a sub-group of patients with non-malignant medical conditions who have severe, intractable pain and who require chronic opioid administration for adequate pain control. Reported here is a systematic clinical evaluation of 52 such patients who were referred after they had failed numerous, non-opioid pain treatments. Major causes of pain were irreversible degenerative and/or traumatic injuries to the musculoskeletal system. A variety of opioids in high daily dosages were required to achieve adequate pain control in these patients. Once pain relief was achieved, patients did not escalate their dosage, and they were able to maintain pain relief for long time periods. Although opioids produced dependence in all patients and complications of constipation and edema in about one third, high daily opioid dosage treatment appeared to be the only medical means to achieve adequate pain control in these subjects. We conclude that opioid maintenance should be utilized as a last resort treatment in patients who fail other pain treatments.

TABLE 1

Major Causes of Chronic Pain
in 52 Patients Treated with Opioids

	No. Patients	%
Arthritis	16	(30.8%)
Post-Trauma	13	(25.0%)
Post-Surgical-Back	11	(21.2%)

Collagen Disease	16	(17.3%)
Post-Surgical-Abdominal	8	(15.4%)
Headaches	7	(13.5%)
Post-Surgical-Cancer	3	(5.0%)
Chronic Pancreatitis	3	(5.8%)
Neuralgia/Neuropathy	2	(3.8%)
Chronic Liver Disease	2	(3.8%)
Gaucher's Disease	1	(1.9%)
Porphyria	1	(1.9%)
Restless Legs Syndrome	1	(1.9%)
Renal Stones-Inoperable	1	(1.9%)
Osteomyelitis	1	(1.9%)
Endometriosis	1	(1.9%)
Gout	1	(2.9%)
Total*	81	

*Numbers add to more than 52 since some patients had more than one major pain source

Table 2

Opioids Administered to 52
Chronic Pain Patients *

Opioid	No. Patients	Usual Dosage Per 24 Hrs(MGS)
Codeine+	18(34.6%)	240-1080
Methadone	14(26.9%)	10-240
Propoxyphene+	7(13.5%)	400-1200
Hydromorphone**	7(13.5%)	8-120
Oxycodone+	5(9.6%)	15-80
Meperidine	4(7.7%)	200-2000
Morphine**	3(5.8%)	60
Hydrocodone+	1(1.9%)	20
Total*	59	

* Adds up to more than 52 since some patients used more than one opioid.

+ These opioids were usually in formulations which contained acetaminophen.

** Subjects used their opioids by suppository only for acute exacerbations of pain.

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The Role of β-Endorphin in Respiratory Disorders in Man

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INTRODUCTION

It is well established that endogenous opioid peptides, such as beta-endorphin and the enkephalins, as well as the more stable analogs of the enkephalins, are capable of producing respiratory depression in experimental animals when administered directly into the brain (Florez et al., 1980; Moss and Scarpelli 1981; Sitsen et al., 1982; Haddad et al., 1984). Specific sites of respiratory depressant action by opioid peptides in the brain have also been identified and include the nucleus tractus solitarius (Hassen et al., 1982), the nucleus ambiguus (Hassen et al., 1984) and the anterior hypothalamus (Pfeiffer et al., 1983). The ability of naloxone to antagonize the respiratory depressant effect of these peptides, as well as the potency characteristics of various opioids, strongly indicated a role of the mu type of opioid receptor in mediating the respiratory depression observed in many of these studies in animals.

The first indication that endogenous opioid peptides may be involved in respiratory depression associated with certain pathological states in humans was in a report by Brandt et al. (1980), who found markedly elevated levels of met-enkephalin immunoreactivity in the brain and cerebrospinal fluid (CSF) of a child with necrotizing encephalomyelopathy. This stimulated a search for increased levels of enkephalins in the brains from victims of the sudden infant death syndrome (SIDS), a syndrome postulated to involve abnormal regulation of ventilation (Shannon et al., 1977). However, abnormal enkephalin levels were not found in the brains of SIDS victims (Kuich and Franciosi 1983; Bergstrom et al., 1984). On the other hand, Orlowski et al. (1982) reported some preliminary data suggesting that infants with apnea had elevated levels of beta-endorphin immunoreactivity (B-EIR) in their CSF. In addition, plasma levels of B-EIR were reported to be significantly increased in infants with apnea of prematurity (Sankaran et al., 1984). This finding has recently been confirmed by MacDonald et al. (1986). These studies indicated the possibility that increased levels of beta-endorphin (or other related opioid peptides) may contribute to the respiratory depression or apnea associated with certain pathological conditions in infants.

The objectives of the present study were to provide confirmatory evidence for elevated levels of B-EIR in CSF from infants with apnea, as well as from infants resuscitated from an apparent life-threatening event (ATLE); to determine whether siblings of SIDS victims had elevated levels of B-EIR in CSF; to determine whether the measurement of plasma B-EIR could serve as a useful index for elevated CSF levels of B-EIR in these patients; and finally, to determine whether increased CSF levels of B-EIR occur in Rett's syndrome, a neurological disorder in females involving respiratory hyperventilation and depression (apnea), seizures, abnormal hand movements and progressive mental deterioration. As part of an ongoing study, we are also assessing the value of treatment with the orally active opioid antagonist naltrexone in children with apnea and in some of the patients with Rett's syndrome who demonstrated elevations in the CSF level of B-EIR.

METHODS

Subjects. Based upon medical history and clinical evaluation, the subjects were assigned to one of the groups described below before any samples were analyzed for beta-endorphin. Group A originally consisted of 2 control subgroups. One was a group of infants admitted to the hospital with suspected apnea (4 subjects) or a history of apnea of prematurity (1 subject), but the presence of apnea was not confirmed by full clinical evaluation (see below). The other subgroup consisted of patients admitted for various other (nonapneic) reasons requiring lumbar puncture (LP) for diagnostic purposes. Results from the analysis of CSF B-EIR in these two subgroups were combined, since there was no significant difference between the mean levels for both groups. Group B consisted of infants with clinically confirmed apnea (see below). The infants in Group C were siblings of SIDS victims, two of which were identical twins of SIDS victims. Only two of the subjects in this group showed a normal pneumocardiogram or polysomnogram during clinical evaluation (see below). Group D consisted of four subjects who had experienced an ALTE (sometimes referred to in the literature as "near-miss" SIDS), requiring active resuscitation (CPR). In one of these, the ALTE had occurred over a year earlier and the CSF was tested because of continued problems with apnea. Another subject in this group died within 24 hours of CSF sampling without regaining consciousness. Group E consisted of patients diagnosed as having Rett's syndrome.

Evaluation. The clinical evaluation of these subjects consisted of a complete clinical examination and laboratory tests, including differential blood count, electrolytes and other blood chemistry, pneumocardiogram, electroencephalogram, and LP. Polysomnography, upper gastroesophageal studies and computerized axial tomography scans were performed when indicated.

Assay of beta-endorphin. A double-blind study was conducted, in which all CSF and plasma samples were coded without identification of the diagnosis, and the clinician was not informed of the results of the analyses until after he had assigned the patients to one of the groups noted above. The lumbar CSF was divided into 1-ml aliquots and frozen immediately. Heparinized blood samples from some of the subjects in Groups A, B and C were obtained between noon and 2 p.m., just after performing the LP. The heparinized blood was centrifuged immediately at 1500 x g for 10 min at 4° C. The resulting plasma was divided into 1-ml aliquots, acidified with 0.1 ml of 1 N HCl and frozen at -70° C until analysis. After thawing, beta-endorphin was extracted from the plasma, using Sep-Pak C-18 cartridges (Waters Associates, Milford, MA), as described by Hong _et al_. (1983). The samples of CSF were not extracted, because the smaller amount of protein in CSF permits the direct assay of beta-endorphin.

Following lyophylization in a vacuum desiccator, the frozen CSF samples were reconstituted in 0.4 ml of 20 mM sodium phosphate buffer, pH 7.5, and divided in half for duplicate determinations of beta-endorphin with a radioimmunoassay kit (Immunonuclear Corp., Stillwater, MN), which utilizes a rabbit anti-beta-endorphin serum. This anti-serum exhibits 100% cross-reactivity with des-Tyr$_1$- and N-acetyl-beta-endorphins, 50% cross-reactivity with beta-lipotropin, but less than 0.1% cross-reactivity with met-enkephalin, leu-enkephalin and dynorphin. After mixing 0.1 ml of the anti-serum with the samples and standard amounts (2.5-1000 pg) of beta-endorphin (Sigma Chemical Co.), the tubes were incubated for 24 hours at 4° C. Following this incubation, 0.1 ml (5 pg) of ^{125}I-beta-endorphin was added to each tube and incubated for another 24 hr at 4° C. Bound and free beta-endorphin were separated using a 2-hr incubation at 4° C with a goat anti-rabbit immunoglobulin precipitating reagent. After centrifugation, the supernatant was carefully removed, and the pellets were counted for 2 min in a gamma counter.

Statistics. Data were analyzed for significant differences with computer programs for Dunnett's test and for linear regression (Tallarida and Murray 1981).

RESULTS

CSF levels of beta-endorphin. The results of the analyses of the CSF samples from each group for B-EIR are summarized in Figure 1. CSF samples from the control group, Group A, had a mean (± S.E.) of 31 ± 3 pg of B-EIR/ml of CSF, which is not significantly different from values in lumbar CSF from adults (Schlachter _et al_., 1983). All of the other groups showed significant elevations in the concentration of B-EIR in CSF. With the exception of 3 of the 20 patients with Rett's syndrome, there was no overlap between the values in CSF from any of the control patients and the individual values from subjects in the other groups.

Comparison of plasma with CSF beta-endorphin levels. From some of the patients in Groups A, B and C in this study, blood samples were obtained after the LP procedure for the purpose of determining whether there was a positive correlation between the levels of B-EIR in plasma and CSF (Fig. 2). This phase of the study

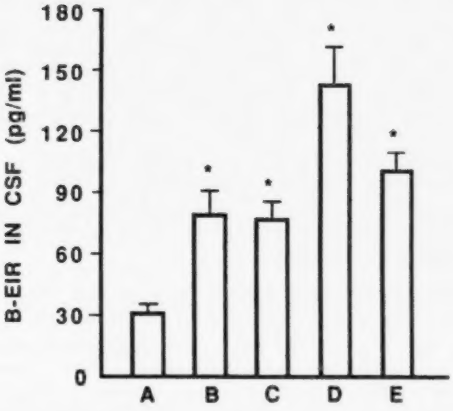

*FIGURE 1. B-EIR in CSF (mean ± SE) from A, 22 Control Infants; B, 10 Infants with Proven Apnea; C, 11 Infant Siblings of SIDS Victims; D, 4 Infants with History of ALTE; and E, 20 Patients with Rett's Syndrome. *P<0.01, compared to Group A (Dunnett's test).*

(i.e., the collection of blood samples) was not continued after linear regression of the results from 11 of the control infants in Group A and from 14 of the infants in Groups B and C indicated the lack of a significant correlation between the levels of B-EIR in plasma and CSF (correlation coefficient = -0.411, N = 25). The individual groups also showed no significant correlation between plasma and CSF concentrations of B-EIR.

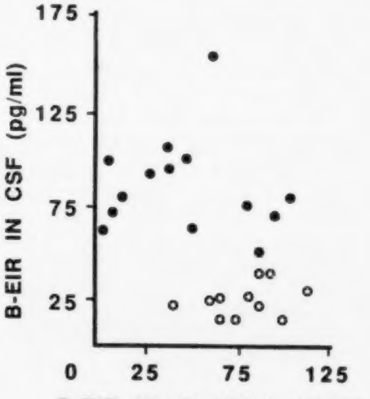

FIGURE 2. CSF vs. Plasma Levels of B-EIR (pg/ml) in Control Infants (open circles) and Infants from Groups B and C (closed circles).

Effects of naltrexone treatment. So far, 19 children with documented apnea of unknown etiology have been treated orally with either naltrexone syrup or tablets (1 mg/kg/day). No side effects of naltrexone treatment have been encountered, other than the precipitation of an opiate-like withdrawal syndrome (hyperactivity, irritability, vomiting and abdominal cramps) within 90 min after the first oral dose in one of the older children.

Of the 12 infants treated with naltrexone, only one had a recurrence of apnea, which coincided with insufficient medication or stress associated with an automobile accident (in which the infant was not injured). Two of the infants treated with naltrexone had repeat LP's (8 mo and 1 yr later), and the B-EIR in their CSF remained elevated.

The 7 older children in our study ranged from 2 to 8 years of age. In addition to apnea, these children also had elevated levels of B-EIR in their CSF (74 to 276 pg/ml). No further apnea was noted during naltrexone therapy. The child with the highest CSF level of B-EIR was a 6-year-old sibling of a SIDS victim and had a history of repeated hospitalization for ALTE's. The first dose of naltrexone precipitated a withdrawal syndrome in this child. The absence of apnea during one month of naltrexone treatment prompted her parents to stop the medication, which was followed by recurrent apnea requiring CPR and reinitiation of naltrexone therapy.

Of the 8 patients with Rett's syndrome who have been treated with naltrexone (1-2 mg/kg/day, p.o.), seizures were decreased in 3, improvement of apnea was observed in 3, and improvements in awareness, responsiveness and behavior were noted in 7 by independent observers. Only one of these patients showed no improvement with naltrexone therapy.

DISCUSSION

The results of the present study indicate that the respiratory depression associated with certain pathological syndromes is accompanied by increased levels of B-EIR in the CSF. Our results from patients with the infant apnea syndrome confirm the findings of a preliminary report by Orlowski et al. (1982) and a more extensive report which appeared while our study was in progress (Orlowski 1986). Sakaran et al. (1986) have also recently reported a significant increase of B-EIR in the CSF of infants who had experienced "near-miss" SIDS within 48 hr of CSF sampling. In addition, we have found increased concentrations of B-EIR in CSF from all of the siblings of SIDS victims and in CSF from most of our patients with Rett's syndrome. Although the infant siblings of SIDS victims were apparently healthy and had not had any apneic events witnessed by their parents, most of them showed an abnormal pneumocardiogram or polysomnogram.

Considering the possible role of endogenous opioid peptides in respiratory function (McQueen 1983; Malcolm and Holaday 1985), these findings indicate that elevated levels of beta-endorphin or related opioid peptides may contribute to the respiratory dysfunction observed in our subjects. This hypothesis is supported by the improvement in respiratory status observed during naloxone infusion (Orlowski 1986) or during therapy with oral naltrexone. The beneficial effect of naltrexone therapy appears to be due to the blockade of opioid receptors, because the patients we have tested during therapy with naltrexone still showed elevated levels of B-EIR in their CSF. Apparently, the levels can be elevated enough to produce a typical opiate-like physical dependence, as indicated by the precipitation of the abstinence syndrome with naltrexone in one of our subjects.

Although it is not known how much of the beta-endorphin released within the central nervous system reaches the CSF at the lumbar level or what fraction of the B-EIR in the lumbar CSF is due to authentic beta-endorphin or other pharmacologically active opioid peptides, the improvement in respiratory function by naltrexone and the precipitation of withdrawal in one patient indicate that at least part of the elevations we observed are due to abnormal increases in endogenous peptides with opioid activity. Therefore, the measurement of B-EIR in lumbar CSF may be a useful marker for deciding to initiate narcotic antagonist therapy or for investigating the possible role of endogenous opioids in SIDS or respiratory disorders accompanying other pathological syndromes. It is not known, for example, whether the increased B-EIR in CSF from siblings of SIDS victims represents a genetic abnormality or is due to environmental factors.

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The Acquired Immunodeficiency Syndrome: Do Drug Dependence and Ethnicity Share a Common Pathway?

L. Brown, Jr., D. Murphy, and B. Primm

INTRODUCTION

Among the problems that continue to plague the drug dependent, the acquired immunodeficiency syndrome (AIDS) has retained a prominent position in the attentions of the scientific and medical communities as well as the lay public. Yet, despite significant technological advances, prevention and health education remain as the greatest prospects for combating the epidemic of AIDS. Efforts directed at known methods of disease acquisition and populations with a disproportionately greater susceptibility may serve as important foci of health prevention. To this end, intravenous drug users and minority group members present some interesting concerns and similarities.

As the second largest hierachical risk group, intravenous drug abusers (IVDAs) represent a significance beyond their own numbers. Intravenous drug use has been associated with 51% of female AIDS cases and 57% of AIDS among pediatric patients (Centers for Disease Control 1986). Furthermore, intravenous drug use is to a large extent responsible for the prominence of reported AIDS cases in the northeastern United States.

While blacks comprise 12% and Hispanics 6% of the United States population (Vital Statistics 1980), their proportion of the reported AIDS cases has been 25% and 14%, respectively (Centers for Disease Control 1986). The racial distribution by risk group also differs between whites and minority group members. AIDS cases that could be traced to homosexual or bisexual men or to the use of blood or blood products were more likely to be white, while blacks and Hispanics predominated in cases associated with heterosexual contacts, with no identifiable mode of transmission or IV drug abuse. Among women, the cumulative incidences for blacks and Hispanics is 13.3 and 11.1 times respectively, the incidence for whites. Of the pediatric AIDS cases for which race/ethnicity information was collected, 58% were black and 22% Hispanic.

A great number of factors might explain these findings, yet investigations of this type are uncommon. To further explore the relationship between ethnicity and IV drug use in connection to the development of AIDS, we present the preliminary findings of an examination of the prevalence of infection with the human immunodeficiency virus (HIV) in association with various patterns of drug use, sexual behavior, medical history and physical examination findings, and various laboratory data.

MATERIALS and METHODS

The Addiction Research and Treatment Corporation, Inc. is a comprehensive drug treatment program with clinics in the New York City boroughs of Brooklyn and Manhattan. Admission is predicated on the demonstration of at least one year of opiate addiction. After informed consent, medical and drug histories and physical examinations were performed by medical staff. The study population was composed of 288 male and 124 female patients applying for admission or currently enrolled in methadone maintenance between the period of January 1, 1986 and March 1, 1986.

Routine laboratory evaluation included a complete blood count (Technicon H 6000), serum electrolytes (Technicon SMAC), liver function tests (Technicon SMAC), and urinalysis (Ames Clinitek 200). Urine toxicology screening was performed by enzyme immunoassay (EMIT) and high performance thin layer chromatography (HPTLC), while hepatitis B was performed by the method of enzyme immunoassay (Abbott). Syphilis serology was assessed by the rapid plasma reagin (RPR) method and serum protein electrophoresis was performed by the microzone method. Sera were also tested in duplicate for HIV antibody by enzyme-linked immunosorbant assay (ELISA) and positives confirmed by Western Blot (Biotech/Dupont) assay.

Statistical data analysis consisted of regression analyses of conceptual domains of variables (see table 1) on seropositivity. Variables from each domain were entered into the equation to determine the total variance for which each accounted. Additionally, the unique variance accounted for by each of the five conceptual domains of variables was determined by assessing the additional variance contributed in turn by each particular domain, once the other four had been entered into the equations.

TABLE 1 REGRESSION EQUATION PREDICTORS

Demographic Domain:	Age, Sex, Ethnicity
Drug Use Domain:	Duration of Drug Use Type of Drugs Most Often Route of Administration
Sexual Behavior Domain:	Preference, Frequency Number of Partners
Medical History/Physical Examination Domain:	Diseases Associated with Drug Abuse Symptoms Physical Findings
Laboratory Data:	Albumin, Globulin Gamma Globulin White Blood Cell Count

RESULTS

The age range of this group was 19 to 62 years with a mean age of 34.4 ±0.35 (SEM) years. The ethnicity of this group consisted of 49 percent black, 42 percent Hispanic, and 9 percent white. The duration of drug use ranged from one year to 38 years with a mean of 12.4 ± 0.33 (SEM) years. The overall prevalence for HIV exposure was 54% with females (see table 2) being slightly more likely than males to have been to be seropositive. Blacks and Hispanics as a group were one and one half times more likely to be positive in this population.

TABLE 2 HIV ANTIBODY PREVALENCE

	N	Positives No. (%)	Odds Ratio
SEX			
Male	288	152 (53)	1
Female	124	71 (57)	1.2
ETHNICITY			
Whites	38	17 (45)	1
Blacks/Hispanics	374	207 (55)	1.53 (1.49-1.58)

From the regression analysis (see table 3), the domains of drug use, medical history and physical examination and laboratory data had a significant total variance. In the drug use domain, this was primarily attributable to those who claimed that the intravenous route was their most often route of administration. In the medical history and physical examination domain, this was largely accounted for by the prevalence of generalized lymphadenopathy. However, only the laboratory data domain had a significant unique contribution to the explained total variance reaching the 0.0001 level of significance.

TABLE 3 TOTAL AND UNIQUE VARIANCES CONTRIBUTED BY DOMAIN OF VARIABLES TO HIV SEROPOSITIVITY

Domain of Variables	Total Variance	Unique Variance
Demographic Domain	0.006	0.005
Drug Use Domain:	0.022 *	0.0008
Sexual Behavior Domain:	0.002	0.007
Medical History/ Physical Examination Domain:	0.04 **	0.02
Laboratory Data:	0.16 ***	0.14 ***

* $p<0.05$ ** $p<0.01$
*** $p<0.001$

DISCUSSION

The acquired immunodeficiency syndrome has brought renewed attention to the consequences of drug dependence. While significant advances have been made on many fronts, the greatest capacity for intervention remains in public education and information. The success of prevention efforts is predicated on tailoring approaches directed at target populations which are either particularly susceptible or which serve as portals for wider dissemination. For this reason, the disproportionate greater occurrence of AIDS and HIV infection among IVDA's and minority group members has begun to stimulate discussion (Bakeman et al., 1986, Centers for Disease Control 1986, Chaisson et al., 1987, Freidland et al., 1985, Ward et al., 1986, Weiss et al., 1985). That IVDAs have a greater seropositivity rate than the general public is understandable; immunologic derangements in IVDAs are not uncommon. In a previous study, we were able to demonstrate that various patterns of drug abuse are associated with greater immunologic aberrancies (Brown et al., 1986).

The racial/ethnic distribution among AIDS cases presents a greater challenge to explain. The present study also demonstrates a greater prevalence of HIV infection among blacks and Hispanics as compared to whites. However, when various groups of drug use patterns, sexual behaviors, medical history and physical examination findings, and laboratory findings were controlled for in a systematic fashion, no demographic variable could explain the distribution of HIV seropositivity in this population. To the contrary, only specific laboratory data that may reflect underlying immunological compromise was significantly associated with exposure to the AIDS virus. Whether this association between the laboratory data studied and HIV antibody prevalence reflects a greater susceptibility as an antecedent to HIV exposure or the consequences following infection can not be explained by this study.

The disproportionate greater frequency of HIV infection among minorities may be in part explained by the maldistribution of intravenous drug use in ethnic minorities. This phenomenon of greater drug use may merely be a reflection of poverty. Economics may also determine various patterns of drug abuse. In this sense, the drug dependent person may more likely practice more risky, HIV exposing behaviors. There may be other, as of yet not fully investigated, cultural factors responsible for the ethnic/racial distribution of AIDS and HIV infection.

There are two basic dividends of this study and subsequent discussion. Blacks and Hispanics are at disproportionately greater risk of developing AIDS or other forms of HIV infection. However, race/ethnicity, by itself does not adequately explain this quite consistent observation by many investigators. It is the prevalence in minority communities of underlying factors, which affords this enhanced risk to HIV exposure and its various sequelae. For blacks and Hispanics, as has been demonstrated in the present investigation and in many other epidemologic evaluations, much of the increased risk can be attributed to intravenous drug use.

Investigations which focus on evaluating racial/ethnic differences in the distribution of underlying, HIV exposing behaviors are in acute need. While basic science and clinical research continue their brisk development, ethnographic studies unlocking information of cultural differences remain center stage in education and prevention efforts. While the public health implications are heightened for ethnic/racial minorities, its significance for society as a whole is undeniable.

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Acute Opioid Physical Dependence in Humans: Naloxone Dose Response Effects

S. Heishman and M. Stitzer

The development of physical dependence is considered to be one causative factor in the continued use and addictive abuse of opioid drugs (Lindesmith 1980). Physical dependence is defined by the appearance of a characteristic abstinence syndrome upon abrupt cessation of a chronically administered opiate (Jasinski 1977). Acute physical dependence (acute antagonist sensitivity) refers to the withdrawal symptoms precipitated by the administration of an opioid antagonist several hours following a single dose of an opioid agonist (Martin and Eades 1961). This phenomenon is particularly interesting given that the opioid abstinence syndrome has generally been thought to develop only after prolonged exposure to opioid agonists.

Studies with both animals and humans have clearly demonstrated the occurrence of acute opioid dependence. Research from the animal laboratory has shown the development of withdrawal symptoms following acute opioid administration with (Aceto et al., 1977) and without (Grilly and Gowans 1986) antagonist challenge. The initial demonstration of acute opioid physical dependence in humans came from the work of Nutt and Jasinski (1974), who observed physiological and subjective withdrawal symptoms precipitated by a methadone-naloxone mixture one week after a single methadone exposure. Consonant with animal studies (Martin and Eades 1964), antagonist precipitated withdrawal symptoms have been reported to be qualitatively similar to those observed in individuals experiencing spontaneous withdrawal from chronic opioid dependence (Jones 1979; Wikler et al., 1953).

Bickel et al. (1986) recently investigated the withdrawal symptoms induced by naloxone 6 hours after single doses of morphine in nondependent humans. Physiological, subjective, and observer rated withdrawal signs were precipitated in a morphine dose related manner, suggesting that the withdrawal response was specifically related to the amount of morphine in the body. The purpose of the present study was to further characterize the acute opioid abstinence syndrome by using a similar paradigm to demonstrate a naloxone dose response function.

METHOD

Subjects

Participants were six males (32-38 years of age) reporting a history of prior opioid use of 5-24 years (mean=13.5) and average current opioid use of 8 times per month. Four subjects reported previous participation in methadone maintenance or detoxification programs; however, none were currently seeking treatment. During the 5-week study, subjects lived on an eight-bed inpatient research unit. In order to verify the absence of current opioid dependence, subjects were observed for withdrawal symptoms for 3-4 days on the research unit and received a challenge naloxone injection prior to the start of the study. No withdrawal symptoms were observed in any of the subjects.

Drugs

The constant morphine dose was either 18 mg/70 kg (N=3) or 30 mg/70 kg (N=3). Naloxone challenge doses were 0.1, 0.3, 1, 3, 10, and 30 mg/70 kg and were presented in random order. Drugs were administered under double blind conditions in a constant volume of 2 ml i.m. in the right or left deltoid muscle.

Procedure

During their first week of participation, subjects were evaluated on two test sessions: 1) placebo morphine followed 6 hours later by a 10 mg/70 kg naloxone challenge, and 2) 18 mg/70 kg morphine followed by 10 mg/70 kg naloxone. Three subjects who failed to report and exhibit naloxone-precipitated withdrawal symptoms following 18 mg morphine pretreatment were tested at 30 mg/70 kg morphine for the remainder of the study. Subjects then participated in seven experimental sessions conducted twice weekly, separated by at least 72 hours. On session days, morphine injections were given at 9:00 am. Five and a half hours after the morphine injection, subjects were seated in a quiet experimental room and connected to physiological recording equipment. While measurements were stabilizing for 15-20 minutes, a baseline pupil photograph was taken, subjective forms completed, and objective signs of withdrawal observed. Baseline physiological measures were then recorded for 10 minutes. Approximately 30 minutes after starting the session (6 hours postmorphine), subjects, who were blind to drug, received an i.m. injection of naloxone or naloxone placebo and were then monitored for 60 minutes. The measurement battery of pupil photographs, subjective reports, and observer-rated objective withdrawal signs was repeated at 5, 15, 30, 45, and 60 minutes postnaloxone.

Physiological measurements

Five physiological measures were recorded each minute throughout the session: (1) systolic and diastolic blood pressure (mm Hg) determined oscillometrically by a Sentron blood pressure monitor with an automatically inflating cuff, (2) mean arterial pressure measured during blood pressure determination by the Sentron monitor, (3) heart rate sampled during blood pressure measurement and converted to beats per minute by the Sentron monitor, (4) respiration rate measured by a chest bellows detecting negative pressure changes which were converted to breaths per minute, and (5) skin temperature measured by a finger thermister. All signals were recorded by and stored on an Apple IIe microcomputer. Pupil photographs were taken in ambient room lighting using a Polaroid camera with 3X magnification. Pupillary diameter was measured in millimeters from the photographs using calipers.

Subjective report measurements

At each measurement point during the test session, subjects completed opioid symptom, withdrawal symptom, and drug effect questionnaires. The opioid and withdrawal symptom forms each contained 15 items describing typical opioid effects (eg., nodding, relaxed, talkative) and withdrawal symptoms (eg., hot or cold feelings, watery eyes, abdominal cramps). The drug effect questionnaire assessed six items: (1) drug "high", (2) any drug effect, (3) good drug effects, (4) bad drug effects, (5) drug liking, and (6) withdrawal sickness. On all questionnaires, subjects rated the extent to which they currently experienced each symptom or effect on a 10-point scale from 0 (not at all) to 9 (most strongly). Items were answered by using a joystick to move a pointer on the computer screen to the desired position of a segmented line. A total score was obtained for the withdrawal and opioid symptom questionnaires by summing across the 15 items; each drug effect question was scored separately.

Observer rated measurements

During the testing session, the experimenter, who was blind to dose and continually present in the experimental room, observed and rated six objective withdrawal signs on the same 10-point scale described above. The following signs were observed: (1) tearing eyes was rated by gently moving the subject's lower eyelid up and down, (2) runny nose was rated by listening for fluid sounds while the subject sniffed, (3) perspiration was rated by the amount of wetness on the subject's palm and forehead, (4) gooseflesh was rated by observing the skin reaction when the observer ran his finger lightly over the inside of the subject's forearm, (5) yawning was rated by observing occurrences during the observation period, and (6) restlessness was rated by observing the frequency of seat movements. A composite observer rating score was obtained by summing the individual item scores.

Data analysis

Data were analyzed by three-way, repeated measures analysis of covariance with morphine dose, naloxone dose, and time postnaloxone as the factors. The prenaloxone baseline score on each measure was used as the covariate. For the continuously recorded physiological measures, baseline scores were an average of the 10 minutes prior to naloxone administration; postnaloxone data were summarized in 5-minute blocks of time. Data from the 18 and 30 mg morphine doses were combined for the present report because pupillary diameter was the only measure which significantly differed between the morphine doses.

RESULTS

Prenaloxone baseline measures (not shown) revealed residual morphine-induced miosis and respiratory depression; pupils were constricted to about 3.0 mm and respirations averaged 15 breaths per minute. As shown for selected measures in Figure 1, naloxone reversed residual morphine effects and precipitated withdrawal symptoms in a dose related manner. Naloxone significantly increased pupillary diameter ($p<.001$) and respiration rate ($p<.001$) over naloxone placebo levels. Heart rate was increased ($p<.001$) and skin temperature decreased ($p<.05$) by naloxone in a dose dependent manner; however, blood pressure failed to show orderly dose related effects. Although not statistically significant, trends in the data of subjective measures suggested a reversal of residual morphine effects. Responses to the "good drug effects", "drug high", and "drug liking" items from the drug effect questionnaire were reversed at doses above 0.3 mg naloxone. Additionally, naloxone precipitated subjective and objective signs of opioid withdrawal. Naloxone produced significant dose related increases in subjective responses on the withdrawal questionnaire ($p<.001$), "bad drug effects" item ($p<.001$), and "withdrawal sickness" item ($p<.01$) from the drug effect questionnaire. Naloxone significantly increased composite observer ratings of objective withdrawal symptoms ($p<.001$); yawning ($p<.01$) and tearing eyes ($p<.05$) were the only individual items showing a significant dose effect.

Time course data over the 60-minute session indicated complete reversal of pupillary constriction by 5 minutes postnaloxone which remained stable throughout the session. Maximum reversal of respiratory depression was evident by 10-15 minutes postnaloxone. Subjective reports and observer ratings indicated that onset of precipitated withdrawal effects was equally rapid. Withdrawal questionnaire scores and composite observer ratings were clearly elevated at 5 minutes with peak effect occurring at 5 or 15 minutes, depending on dose. Responses on both measures gradually declined to placebo levels within the session, except for the 30 mg naloxone dose scores which remained elevated at 60 minutes postnaloxone.

DISCUSSION

This study documented the occurrence of antagonist precipitated withdrawal symptoms in humans following single opioid exposures and extended prior work by demonstrating naloxone dose response effects.

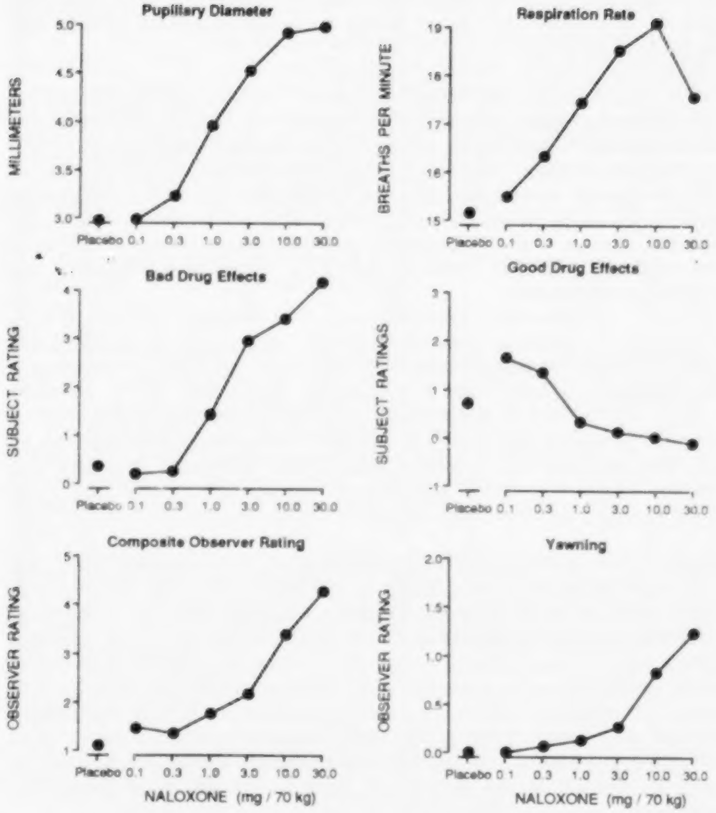

FIGURE 1. Effects of naloxone dose on selected physiological (top panels), subjective-report (middle panels), and observer-rated (bottom panels) measures. Pupillary diameter, subjective-report, and observer-rated data for each naloxone dose are means of measurements taken 5, 15, 30, 45, and 60 minutes postnaloxone. Respiration data are means of 12 5-minute time blocks. Data are adjusted for prenaloxone baseline values and show means of six subjects.

Subjective responses on a withdrawal symptom questionnaire, on "bad drug effects" and "withdrawal sickness" ratings, and composite observer ratings of withdrawal were significantly increased by naloxone in a dose dependent manner. Naloxone simultaneously produced significant dose related increases in pupillary diameter and respiration rate, indicating the reversal of residual morphine effects. These findings, together with the work of Bickel et al., (1986) who reported morphine dose related effects using a similar paradigm, suggest that precipitated withdrawal is specifically related to the presence of opioid agonist and antagonist in the body.

With the exception of respiration rate which showed a decrease at the 30 mg naloxone dose, physiological, subjective report, and observer rated measures revealed an orderly dose related progression to the highest naloxone dose. This suggests that the underlying mechanism of acute antagonist sensitivity may be dependent upon the degree of receptor occupancy by agonist drug which was not maximally reversed by naloxone in this study. It would be interesting to test higher naloxone doses to clarify the upper end of the dose response relationship for withdrawal precipitation.

The reversal of residual morphine effects and onset of precipitated withdrawal symptoms occurred rapidly following naloxone administration; effects were generally apparent by 5 minutes postnaloxone. This is consistent with naloxone's rapid onset in reversing the effects of opioid overdose. The similar time course for opioid reversal and withdrawal precipitation suggests that the two processes share the same mechanism, displacement of opioid drug from the receptor site.

Consonant with previous studies (Bickel et al. 1986; Jones 1979), the reported and observed precipitated withdrawal symptoms were similar to those reported by individuals experiencing spontaneous withdrawal from chronic opioid dependence. Yawning was the most obvious objective sign. This similarity suggests that the development of opioid physical dependence is a continuous process beginning with the first exposure to opiates. Because physical dependence can perpetuate opioid use and abuse (Lindesmith 1980), examining the full range of its developmental pattern is essential to a complete understanding of the etiology of opioid abuse and the formation of effective treatment approaches.

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Behavioral, Physiological and Hormonal Effects of a Naloxone Challenge Following Acute Morphine Pretreatment in Humans

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The administration of an opioid antagonist (e.g., naloxone) following chronic treatment with an opioid agonist (e.g., morphine) produces a withdrawal syndrome. Less well defined is the phenomenon referred to as "acute physical dependence" in which the administration of an opioid antagonist shortly after a single dose of an opioid agonist also produces a withdrawal syndrome. Acute physical dependence of the morphine type has been characterized in several nonhuman species (e.g., rat, monkey, dog) using a variety of unconditioned and conditioned behavioral responses as well as physiological and neuroendocrine measures (e.g., Eisenberg, 1982; Eisenberg & Sparber, 1979; Krystal & Redmond, 1983; Martin & Eades, 1964; Smitts, 1974; Sparber et al., 1978). Acute physical dependence also has been demonstrated to occur in humans, but has not been well characterized (Bickel et al., 1986; Jones, 1980, Nutt & Jasinski, 1974). For example, the only dependent measures reported to date are composite ratings of opioid withdrawal and routine physiological measures (e.g., respiration, pupil size). Thus, it remains to be determined to what extent acutely precipitated opioid withdrawal in humans is similar to the more thoroughly studied withdrawal syndrome observed following chronic administration of opioid agonists. The present study was conducted to further characterize in humans the effects of a naloxone challenge following acute treatment with varying morphine doses. The procedure also obtained a more comprehensive evaluation of acute physical dependence by including behavioral and neuroendocrine measures in addition to the standard signs and symptoms of opioid withdrawal.

METHOD

Subjects

Six male postaddict volunteers who were current sporadic users of illicit opiates participated in the study. Subjects gave their informed written consent to participate and resided on the residential research unit of the Addiction Research Center (ARC) for the duration of the study. To verify the absence of physical dependence, subjects were required to provide a drug-free urine specimen on the day of admission to the ward. Further, all subjects were required to reside on the ward, without opioid exposure, for 10 days prior to the start of the study. None of the subjects exhibited signs or symptoms of withdrawal during this 10-day pre-study period.

Drugs

During each experimental session, subjects received an injection in the morning (A.M.) followed 6 hours later by a second injection (P.M.). Subjects were exposed once to each of the following dose conditions (A.M. & P.M. drugs are listed first and second, respectively, for each dose condition): (1) placebo, placebo; (2) placebo, 10 mg naloxone; (3) 4 mg morphine, 10 mg naloxone; (4) 8 mg morphine, 10 mg naloxone; (5) 16 mg morphine, 10 mg naloxone, (6) 16 mg morphine, placebo. Subjects always received dose-condition 1 first; the remaining 5 dose conditions were scheduled according to a latin square design. This design permitted days 1 and 2 to serve as vehicle control days. Dose condition 6 permitted observation of the time-course of morphine (16 mg) in the absence of a naloxone challenge. Finally, dose conditions 3-5 permitted assessment of naloxone precipitated opiate withdrawal.

Procedure

Experimental sessions were conducted in two components, A.M. and P.M., which are described below:

A.M. component: The subject was escorted to the experimental room and an intravenous catheter was inserted in his nonpreferred arm. Physiological measures (respiration, heart rate, blood pressure, skin and oral temperature, and pupil diameter) were permitted to stabilize for 30 min, and thereafter were assessed predrug and 15, 30, 45, 60, 90, and 120 min postdrug. Subject ratings were recorded during the same observation times as the physiological measures, except for ratings of "drug effect" which were assessed only postdrug. The Digit Symbol Substitution Task (DSST) was completed at predrug and 45 and 90 min postdrug. Blood samples were drawn predrug and 15, 30, 60 and 120 min postdrug. Following the 120 min postdrug observation, subjects returned to the ward for 3.25 hrs before beginning the P.M. component.

P.M. component: Physiological measures were stabilized for 30 min and then recorded predrug and 10, 30, 45, and 60 min postdrug. Subject ratings were recorded at these same observation times, except ratings of drug effect which were assessed only postdrug. The DSST was recorded predrug and 10, 30 and 60 min postdrug. Blood samples were drawn predrug and 15, 30, and 60 min postdrug.

Subject-Rated Measures

Subjects rated drug effects on 100 point visual line analog scales marked at either end with "not at all" and "exremely," in both the A.M. and P.M. components. Scales included "strength of drug effect," "good" drug effects, "bad" drug effects, "drug liking," "high," and "sick." Other scales were also used to measure possible symptoms of opiate agonist and opiate withdrawal effects. The opiate withdrawal questionnaire consisted of true/false questions drawn from the ARC Inventory Weak Opiate Withdrawal Scale.

Prolactin and Cortisol Levels

Prolactin and cortisol levels were determined from blood samples which were collected as described above. Plasma samples were analyzed using standard radioimmunoassay techniques.

Data Analysis

Effects in the A.M. and P.M. components were analyzed separately. All measures except subject ratings of drug effect were analyzed as changes from predrug values.

RESULTS

Subject Ratings

A.M. component. Ratings in the A.M. component were consistent with the characteristic acute effects of opioid agonists in postaddict subjects. For example, scores on the MBG scale (i.e., "euphoria") of the ARC

Inventory and visual-analog ratings of drug "liking" and "high" increased as an orderly function of morphine dose (P <0.001).

P.M. component. In the P.M. component, subjects reported symptoms characteristic of opiate withdrawal. For example, the naloxone challenge increased scores on the withdrawal questionnaire on days that subjects were pretreated with 8 and 16 mg of morphine, and a slight increase was observed following the 4 mg morphine dose (P <0.02). Additionally, subject ratings on several of the visual-analog scales increased (e.g., sick, restlessness) as an orderly function of morphine pretreatment dose (P <0.05).

Behavioral Measures

Neither speed nor accuracy measures on the DSST were affected in the A.M. or P.M. components under any of the dose conditions.

Physiological Measures

A.M. component. Consistent with the characteristic effects of opioid agonists, pupil diameter and respiration decreased in the A.M. component as a function of increasing morphine dose (P <0.001). No other physiological measures were affected during this component.

P.M. component. The P.M. naloxone challenge reversed the pupillary effects and also increased heart rate following the 8 mg and 16 mg morphine pretreatment doses (P <0.03).

Neuroendocrine Measures

A.M. component. The 8 and 16 mg morphine doses increased prolactin levels in plasma, while the 4 mg dose and placebo produced no effect and small decreases, respectively (P <0.001). Cortisol levels decreased across all of the dose conditions, but the decreases observed after 16 mg of morphine were of greater magnitude than those observed under the control conditions (P <0.01).

P.M. component. Prolactin levels decreased in the P.M. component as an orderly function of morphine pretreatment dose (P <0.001). This effect was not related to the naloxone challenge, however, since similar decreases were observed when naloxone or placebo followed the 16 mg morphine dose. Naloxone increased cortisol levels following the three morphine pretreatment doses and also the placebo pretreatment dose (P <0.003); however, the increase in cortisol levels following the 8 mg morphine dose was of greater magnitude than the increase observed following the placebo pretreatment condition.

CONCLUSIONS

The present results further characterize the effects of naloxone administration following a single dose of morphine. These findings extend those of earlier studies in which it has been demonstrated that this procedure can produce signs and symptoms qualitatively similar to those observed during abrupt abstinence from a schedule of chronic opioid administration. Specifically, increases in subject ratings of discomfort and increases in pupil diameter and heart rate were observed. Additionally, the present study is the first to demonstrate in humans that such a procedure produces elevated plasma cortisol levels. The failure to find significant effects on the behavioral measure (DSST) indicates that, at the drug doses examined in the present study, subjects remained capable of performing this complex task despite feeling sick, etc.

In the A.M. component, subject ratings of drug effect, physiological measures, and neuroendocrine measures changed as an orderly function of morphine dose. The withdrawal-like effects in the P.M. component also were roughly dose dependent; effects observed following 8 mg and 16 mg morphine pretreatment were reliably greater than those produced by the 4 mg dose. It is noteworthy, however, that effects in the P.M. component following the 8 mg pretreatment dose sometimes exceeded those observed with the 16 mg dose. This overall difference is largely attributable to data from one subject who experienced unpleasant side effects (e.g., vomitting) during the A.M. component from the 16 mg dose of morphine; those unpleasant effects were reduced during the P.M. component by the naloxone challenge. In contrast, the 8 mg pretreatment dose did not induce such side effects and, when a naloxone challenge followed, this subject reported an increase in withdrawal symptoms. Overall, then, effects in the A.M. and P.M. components generally were an orderly function of morphine pretreatment dose, but complex interactions were evidenced in the later component depending on the degree of side effects associated with the agonist dose.

The present results extend the conditions under which acute withdrawal from opioids has been studied in humans. These results confirm earlier findings of a high degree of concordance between the withdrawal syndromes observed when an antagonist challenge is administered following acute and chronic agonist pretreatment. These findings suggest that the acute physical dependence model holds promise as a practical method for investigating factors that influence the development and maintenance of physical dependence. This procedure could lend itself to studies in humans of issues not as readily testable using conventional models of physical dependence. For instance, it is plausible that similar tests could be conducted in subjects without histories of opioid abuse.

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Drug Discrimination in Human Post-Addicts: Agonist/Antagonist Opioids

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The drug discrimination paradigm has been used extensively in animals to study the stimulus properties of the opioids. Our laboratory has developed an experimental paradigm modeled after that used in the animal laboratory to the study the discriminative stimulus properties of the opioids in human opioid abusers. This human drug discrimination paradigm combines the advantages of laboratory drug discrimination methods with those of traditional clinical psychopharmacology methods. As with laboratory drug discrimination methods, subjects receive explicit behavioral training to discriminate standard training drugs against which to compare test compounds or test doses. As with clinical psychopharmacology methods, subjects provide subjective reports concerning the drugs' effects. We have previously reported the results of two studies, one in which opioid-dependent volunteers were trained to discriminate between the effects of saline, hydromorphone, and naloxone (Preston et al., 1985) and a second study in which non-dependent opioid abusers were trained to discriminate between the effects of saline, hydromorphone, and pentazocine (Bickel et al., 1986). In each of these studies, subjects easily learned to discriminate between the training drugs. Generalization testing with various doses of the training drugs resulted in dose-related increases in drug-appropriate responding and in those subjective effect measures which had been shown previously to be sensitive to the training drugs.

The present study was a follow-up to the second study and was designed to assess the stimulus properties of several opioid mixed agonist/antagonists. Post-addict volunteers were trained in a three-way discrimination between saline, hydromorphone, and pentazocine. Generalization curves were then determined for various doses of the active training drugs and three agonist/antagonist opioids, butorphanol, nalbuphine, and buprenorphine. Dependent variables included three different measures of discrimination, as well as more traditional self-report and physiological measures.

METHODS

Subjects: The participants were six adult male post-addicts who gave written informed consent and who were paid for their participation. The subjects reported prior narcotic addiction of 15 to 26 years duration. Prior to their participation, subjects were found to be in good health and without significant psychiatric disturbance other than their drug abuse. Subjects participated while residing on an eight-bed behavioral pharmacology research ward.

Drugs: The training drugs were normal saline (4 ml), hydromorphone 3 mg/70 kg, and pentazocine 45 mg/70 kg. Dose-response generalization testing was conducted on the two active training drugs, hydromorphone and pentazocine, and butorphanol (0.75, 1.5, 3, 6 mg/70 kg), nalbuphine (3, 6, 12, 24 mg/70 kg) and buprenorphine (0.11, 0.22, 0.45, 0.9 mg/70 kg). Commercially available preparations of each drug, diluted to a constant volume of 4.0 ml, were given intramuscularly under double-blind conditions in two divided doses (2 ml in the right and left deltoid muscle). Training drugs were identified to subjects only by arbitrary letter codes. For each subject the drug letter codes associated with each of the training drugs were randomly determined, but remaired unchanged throughout the protocol

General Methods: The study proceeded in three phases, with sessions conducted daily. Discrimination training was conducted in sessions 1-6, during which the subject received two sessions of exposure to each of the three training drugs (saline, hydromorphone and pentazocine). During these training exposures each drug was identified to the subject by letter code prior to drug administration. The subject was instructed to attend carefully to the drug effects and to try to discriminate precisely among them; he was informed that in each session he would earn money by correctly identifying the administered drug by letter code. In sessions 7-12 acquisition of the discrimination was tested by exposing the subject to the training doses of each of the training drugs twice in randomized block order to determine whether the subject could correctly identify the training drug/doses by letter code. During these and all subsequent exposures to the training doses the subject received feedback about the code of the administered training drug after the session. This test-of-acquisition procedure was also repeated among test sessions during the subsequent testing phase to provide continued training and to insure continued correct discrimination. Beginning with session

13, a series of test sessions was conducted. Test sessions were randomly interspersed with test-of-acquisition sessions in which one of the training drugs/doses was given. During this testing phase dose response curves for each active training drug were determined in randomized order, followed by dose-response curves for butorphanol, nalbuphine, and buprenorphine. Following each test session the subject did not receive feedback about the correct drug identification but was informed that it had been a test session and that the drug code could not be revealed. Earnings in training and test-of-acquisition sessions were calculated based on correct identifications in each of the discrimination measures; earnings on test days were not contingent on subject responses but were based on the average amounts earned in previous test-of-acquisition sessions.

Experimental Session: A microcomputer presented all questionnaires in a prearranged and timed sequence. The subject indicated his responses on manipulanda consisting of a numeric pad and three telegraph keys. Daily sessions began with the measurement of respiration, heart rate, temperature, blood pressure and pupil diameter and baseline self-report questionnaires. Drug was then given by the nursing staff. During the initial training sessions the subject was informed of the drug's identifying letter code at the time of injection. The subject remained under observation for 20 min, and then returned to the experimental room to complete the post-drug measures. Post-drug testing consisted of 4 repeating cycles which began at 20 min intervals. Each cycle contained assessments of subjective effects; drug discrimination questions were presented twice, once in the third cycle and again in the fourth cycle. At the end of the session the staff again recorded physiological measures. A sealed envelope was then opened, and the staff informed the patient of the letter-code identity of the administered drug or that the session had been a test session, and the amount of his earnings.

Discrimination Measures: Drug discrimination data were collected in three ways. In each procedure only correct responses were converted to monetary reinforcement for the subject. As one component of each assessment cycle the subject made a discrete choice, naming by letter code (A, B, or C) the drug he thought he had received. To gain quantitative information concerning the degree of stimulus similarity or concerning the subject's confidence in his discrete choice, in a second component the subject distributed 50 points between one or more of the three drug choice alternatives depending upon how certain he was of the identity of the administered drug. In a third component the subject responded on a fixed interval 1 sec schedule on computer keys designated with drug letter codes to earn points for 8.5 minutes. Points could be earned for each of the three choice drugs by pressing the key corresponding to that drug with a 10-sec delay occurring whenever the subject switched keys.

Subject-Rated Measures: Questionnaires included: (1) 100-point quantitative visual analog scales to indicate the degree of drug effects, drug liking, "good" and "bad" effects, subjective "high", and the similarity of the drug effect to each of the training drugs on a scale from "not at all" to "extremely"; (2) a 32-item adjective rating questionnaire containing an opioid Agonist scale (to detect traditional mu-receptor agonist effects), an Antagonist scale (to detect opioid withdrawal effects), and a scale composed of side-effects reported for the mixed agonist-antagonist opioids (kappa/sigma receptor agonists), and (3) a 49-item shortened form of the Addiction Research Center Inventory (ARCI), which contained the MBG ("euphoria"), LSD ("dysphoria") and PCAG ("sedation") scales.

Data Analysis: The results of the discrimination training (discrimination of the training doses following two exposures to each training drug) are reported as mean percent correct identifications over the entire session from sessions 7-12. The results of the subjective measures from the training and test-of-acquisition phase are reported as the mean of scores from 4 exposures to each drug in sessions 1-12. The data reported for the subjective effects are those collected at the end of the fourth cycle, approximately 110 min post-injection. Results of the generalization testing for discrimination measures and subjective effect measures were analyzed as the mean of scores for one exposure to each drug condition. Repeated measures analyses of variance were conducted on the training and test-of-acquisition phases and on the dose response functions for each active drug. Effects were considered statistically significant if $p \leq 0.05$.

RESULTS AND DISCUSSION

The discrimination between the training drugs was readily learned, and few errors were made in identifying the training doses during test of acquisition sessions 7-12. Saline and hydromorphone 3 mg were correctly identified in 100% of these trials; pentazocine 45 mg was correctly identified in 75% of trials. When errors occurred, they typically occurred identically on all discrimination measures. The effects on subjective effects measures produced by the training drugs in sessions 1-12 were similar to those previously reported. Hydromorphone and pentazocine produced significant increases in the drug effect, high, liking, and good effects quantitative visual analog scales compared to saline. On the three adjective rating scales hydromorphone produced the largest increase in Agonist scale scores while pentazocine produced the greatest increase in Antagonist scale scores. The Mixed Agonist/antagonist scale scores were increased approximately equally by hydromorphone and pentazocine. On the ARCI pentazocine produced a significant increase in LSD scale scores. No statistically significant effects on any of the other ARCI scales were found, though hydromorphone tended to increase MBG scale scores. On the three visual analog scales which asked subjects to rate "How much is this drug like ... (each of the training drugs)? each training drug was rated as being very similar to itself, with very little similarity to the other training drugs. Hydromorphone and pentazocine produced a decrease in pupil diameter and an increase in systolic blood pressure compared to saline.

During generalization testing similar results were found on all three discrimination measures. Hydromorphone produced dose-related increases in identifications as hydromorphone and dose-related decreases in identifications as saline with the two lowest doses of hydromorphone being identified as pentazocine in approximately 50% of trials. On the visual analog scales measuring the similarity of the test drug to each of the training drugs hydromorphone produced dose-related increases in ratings of similarity to hydromorphone 3 mg; however, even the 3 mg dose was rated as being only approximately half the maximum scale of similarity to itself. This is in contrast to the 100% hydromorphone-appropriate responses on the operant discrimination measure at the 3 mg dose. Hydromorphone produced significant increases in the Agonist adjective rating scale, the drug effect, high, liking, and good effects visual analog scales, and the MBG scale of the ARCI. Pentazocine produced dose-related increases in identifications as pentazocine and dose-related decreases in identifications as saline. Pentazocine produced very few identifications as hydromorphone. On the visual analog scales measuring the similarity of the test drug to each of the training drugs pentazocine produced dose-related increases in ratings of similarity to pentazocine 45 mg; however, as with hydromorhphone, even the 45 mg training dose was rated as being only approximately half the maximum scale of similarity to itself. Pentazocine produced significant increases in the drug effect, high, liking, and good effects visual analog scales and in the LSD scale of the ARCI.

Butorphanol produced dose-related increases in identifications as pentazocine and dose-related decreases in identifications as saline. On the visual analog scales measuring the similarity of the test drug to each of the training drugs butorphanol produced dose-related increases in ratings of similarity to pentazocine 45 mg.

Butorphanol tended to increase the Agonist adjective rating scale and produced significant increases in the Antagonist and Mixed Agonist/antagonist adjective rating scales, the drug effect, high, and bad effects visual analog scales and in the LSD scale of the ARCI. The effects of nalbuphine did not clearly show it to be similar to either pentazocine or hydromorphone. Nalbuphine 3 mg produced primarily pentazocine-appropriate responses while 6 mg produced primarily hydromorphone-appropriate responses. The two highest doses were identified approximately equally as hydromorphone and pentazocine. None of the doses were identified as being saline. On the visual analog scales measuring the similarity of the test drug to each of the training drugs nalbuphine was rated as being not very similar to any of the training drugs. On most of the subjective effect measures nalbuphine produced relatively flat dose-response functions and produced significant increases in only two measures, the Antagonist adjective rating scale and the drug effect visual analog scale. Buprenorphine also was not clearly demonstrated to be similar to either pentazocine or hydromorphone. The three highest doses were identified equally as hydromorphone and pentazocine, and only the lowest dose was ever identified as being saline. On the visual analog scales measuring the similarity of the test drug to each of the training drugs buprenorphine was rated as being more similar to pentazocine than to hydromorphone, particularly at the highest dose. Buprenorphine tended to increase scores on all of the adjective rating scales and produced significant increases in the drug effect and good effects visual analog scales and in the MBG and LSD scales of the ARCI.

In conclusion, the results of this study replicated an earlier study (Bickel _et al_., 1986) showing that post-addict volunteers could be trained to discriminate reliably between the administration of saline, hydromorphone, and pentazocine in a study paradigm analogous to that used in animal drug discrimination studies. Generalization testing with novel doses of the active training drugs produced dose related increases in drug appropriate identifications. Further, as found in previous studies (Preston _et al_., 1985; Bickel _et al_., 1986) the results indicated that there were no differences among the three measures of drug discrimination used in this study. The effects of the test compounds on the discrimination measures and subjective effects measures were generally in concordance. Butorphanol produced a pattern of changes in subjective effect measures which was similar to that produced by pentazocine and was clearly identified as being like pentazocine in the discrimination measures. Nalbuphine had only moderate effects on the subjective measures and, while clearly identified as not being saline, was not strongly identified as hydromorphone or pentazocine. Buprenorphine also did not produce a pattern of subjective effects similar to hydromorphone or pentazocine and was not clearly identified as being hydromorphone- or pentazocine-like. The drug discrimination measures appear to provide an additional useful tool with which to study the effects of the opioid agonist/antagonists.

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Hormone Secretion in Methadone-Dependent and Abstinent Patients

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INTRODUCTION

This paper presents results from a project that was designed to study the relationship between hormones and dysphoric affects in methadone maintained and detoxified opiate addicts. The data presented here represent the hormonal changes observed; the affective changes will be reported elsewhere.

Acute administration of opiates has been observed to decrease serum testosterone and LH levels (Mirin et. al.,1976), and to increase prolactin and inhibit secretion of cortisol (Zis et. al.,1984). Chronic administration of opiates, as occurs during methadone maintenance, has been reported to produce more variable effects. Cushman and Kreek (1974) reported no significant differences in plasma testosterone, prolactin, FSH and LH between normals and 8 selected stable male methadone patients, though there was a tendency for those with lower plasma methadone levels to have higher testosterone levels. Conversely, Mendelson et. al., (1975) reported suppression of testosterone in addicts maintained on 80-150 mg. methadone/day while finding no differences from normals in patients receiving 10-60 mg./day. Azizi et. al.,(1973) found suppression of testosterone but not LH in 22 male subjects addicted to heroin or methadone. Cushman et. al., (1970) found that morning plasma corticosteroid levels were within the normal range for methadone-treated heroin addicts; afternoon levels were high and 62.5% of the patients studied did not have a normal diurnal variation. Renault et. al.,(1972) also found both normal morning and afternoon cortisol levels in five methadone-treated heroin addicts. In Renault's study, methadone was administered at noon, and close examination of the data suggests that the morning plasma cortisol levels were higher than those found in the afternoon. These somewhat inconsistent findings may relate to a number of factors such as dose, the length of time on methadone, and the time at which plasma samples were taken in relationship to the time of the last methadone dose.

Findings following detoxification from narcotics have been more consistent. Eisenman et. al. (1960) found "tremendous increases" in both plasma and urinary 17-OHCS immediately after withdrawal in two subjects who had become experimentally addicted to morphine. It was not clear how long these levels remained elevated. While studying testosterone levels in methadone maintained opiate addicts, Mendelson and Mello (1975) found a return of the suppressed levels observed during methadone maintenance to normal about one month after detoxification.

The study reported here attempts to expand upon the available data in this area, and to clarify some of the discrepancies noted above, by examining the secretion of testosterone, LH, cortisol and prolactin in independent samples of opioid addicts during well-defined stages of maintenance, detoxification and abstinence, and at multiple points in time.

METHODS

Subjects

Male heroin addicts between the ages of 19 and 35, meeting FDA requirements for methadone maintenance and who were free of serious medical or psychiatric disorders were recruited. Subjects who fit into a variety of categories relative to their time on methadone were chosen, as seen in Table I. The methadone dose for all subjects on maintenance was .9mg./kg. (+ or - 10 mg.) except the pre-maintenance group ("off the street") who were tested immediately upon beginning maintenance treatment and before they had reached their final maintenance dose.

TABLE 1

OCCASION #	DESCRIPTION	# SUBJECTS
1 PRE-MAINTENANCE ("Off-the-Street")	New intakes on no more than 30mg methadone, for not longer than 2 weeks.	18
2 EARLY MAINTENANCE	Not on methadone longer than 2 weeks, after reaching a dose of .9mg/kg body weight (+/- 10mg)	27
3 STABLE MAINTENANCE	4-6 months on .9mg/kg body weight (+/- 10mg)	27
4 LONG TERM MAINT	On methadone 2 or more years, at .9mg/kg +/-10mg.	10
5 EARLY DETOXIFICATION	Immediately post detoxification: 2-5 days after last methadone dose.	18
6 EARLY DRUG FREE	3 or more weeks post detoxification.	9
7 LONG TERM D.F.	No methadone or illicit drugs for 2 or more years.	15
8 CONTROLS	Non-addicts matched for age, race, sex and socioeconomic status.	16

All maintenance subjects were recruited from the drug treatment program at the Philadelphia V.A. Medical Center. Controls were members of the hospital staff who were drug-free and had a negative history for drug abuse. Long term drug-free subjects were recruited from graduates of two nearby therapeutic communities. Both the long term drug-free and control groups were matched by age, race and other sociodemographic characteristics with the maintenance groups. All the long term drug-free subjects had been treated with methadone for at least six months during their addiction career. The medical history, physical examination, SMA 6/12, urinalysis, and urine drug screen were used to verify the absence of current drug use and other serious medical or psychiatric disorders in all subjects tested. Contacts from the therapeutic communities also served to verify the fact that the long term drug-free subjects were legitimate members of that category. Subjects were excluded if they were taking any medication other than methadone.

Procedure

Study subjects were admitted to the Philadelphia V.A. Medical Center inpatient drug ward for testing. The procedures followed on all testing occasions were identical except for methadone administration. Informed consent was obtained from each subject prior to study, according to standard procedures.

Blood samples were obtained by means of a Sigmamotor microflow blood withdrawal pump through an indwelling non-thrombogenic catheter. The pump, which provides an integrated plasma sample, was set at 10 ml./hr. and collecting tubes were changed at 30-minute intervals. All blood samples were centrifuged immediately after completion of collection and the plasma was separated and stored at -25 degrees centigrade until analyzed. Hormone analyses were done by SmithKline laboratories.

Blood samples were collected every half hour over a 7 hour period (8:30 A.M.- 3:30 P.M.) for determination of plasma testosterone, LH, cortisol and prolactin. Methadone patients were given their daily dose at 10 A.M. Methadone patients had been in the ward for at least the prior 24 hours, thus all had received at least two consecutive daily doses at 10 A.M., and were free of illicit drugs. Controls and the long term drug-free subjects rested at home overnight and entered the inpatient drug ward at 7:30 A.M. on the day of testing.

RESULTS

Maintenance

The levels of the four hormones studied on each occasion are seen in Tables 1-4A (maintenance groups) and 1-4B (drug-free groups). All maintenance subjects demonstrated suppression of cortisol, testosterone and LH, beginning one and one half hours following the daily oral dose. Prolactin levels rose precipitously at about the same time. All hormone levels appeared to slowly revert toward those observed in the normal subjects beginning approximately three to four hours after the daily methadone dose, such that they approached control levels by the end of the 24 hour methadone dosing cycle. However, this return toward normal levels was not identical for all hormones on all occasions. As seen in the tables, the levels of cortisol, testosterone and LH appear to be suppressed less in the long-term maintenance patients (more than 2 years on methadone) than in the "off the street" and early maintenance groups. Similarly, the rise in prolactin levels appears to be greater in the early maintenance patients than in the long-term group. These differences between occasions suggest that partial, but not complete, tolerance to the hormonal effects of methadone occurs.

Drug free

There was an immediate and marked rise in cortisol levels immediately after detoxification with a return to normal in the long-term drug-free subjects. This elevation in cortisol was present even 2-3 weeks following detoxification, though to a lesser degree than at 2-5 days. Prolactin levels returned to normal immediately after detoxification, however the long-term drug-free patients had significantly higher levels of prolactin than any other of the drug-free groups. There was an immediate rise in testosterone and LH after detoxification. The levels observed on Occasion 5 (Early detoxification) appear somewhat higher than controls, with a return to control levels by 2-3 weeks. This change is not substantial, however, the long-term drug-free subjects had significantly higher LH and testosterone levels than the controls, as they have with prolactin.

DISCUSSION

These data indicate that discrepancies in earlier data about the effect of methadone on these hormones can be accounted for by taking into consideration 1) the time at which the hormone is measured relative to the daily methadone dose and 2) the length of time that the person has been on methadone. For example, all hormones more closely approach normal levels if measured immediately before the daily dose, and if measured at this point one is more likely to find that they do not differ from normals. However, significant differences are usually observed if the same measurements are done three or four hours after dosing. Differences in hormonal levels can also be masked, or accentuated, by choosing subjects who have been on methadone for varying periods of time. We do not have data on differences according to dose as we attempted to hold dose constant on all occasions except #1, but Mendelson et. al's (1974) findings indicate that dose can also significantly effect the results obtained.

The finding of elevated testosterone, LH and prolactin levels in the long term drug-free subjects is interesting. We cannot be certain if this reflects a rebound from the suppression of these hormones while on methadone, or if it is a function of the sample. Most heroin addicts do not achieve long term abstinence, and thus these subjects may differ from the maintenance patients in terms of motivation, social skills or other dimensions. On the surface this group resembles all the others, including having been treated with methadone for at least six months at some time in their lives. However they may be significantly different from the others in some subtle and perhaps difficult-to-measure ways.

Finally, it should be noted that we began this study in an attempt to explore possible biological alterations in a group of patients with high levels of psychiatric disturbance (Rounsaville et. al, 1982). Our data confirms the fact that methadone has potent endocrinologic effects, accentuating diurnal secretion in some hormones while significantly altering the secretion of others. The behavioral and physiologic implications of these alterations, and their long-term sequelae, may be clinically significant.

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TABLE 1A. CORTISOL LEVELS BY TIME AND GROUP

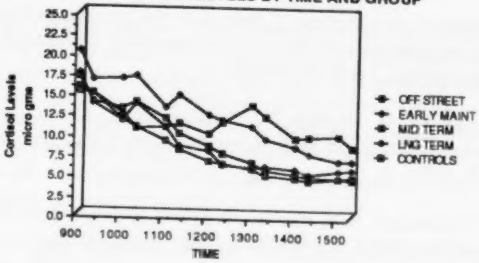


TABLE 2A. PROLACTIN LEVELS BY TIME AND GROUP

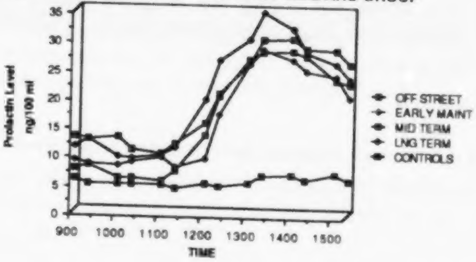


TABLE 3A. TESTOSTERONE LEVELS BY TIME AND GROUP

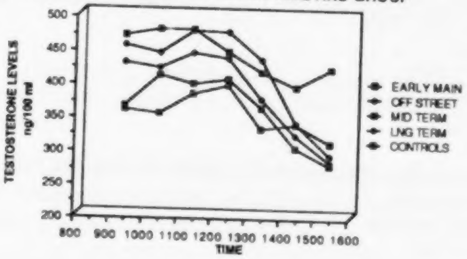


TABLE 4A. LH LEVELS BY TIME AND GROUP

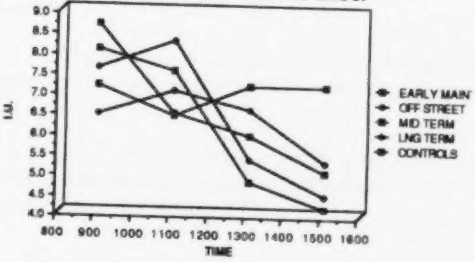


TABLE 1B. CORTISOL LEVELS BY TIME AND GROUP

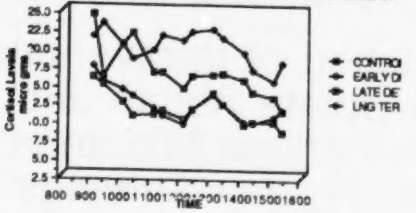


TABLE 2B. PROLACTIN LEVELS BY TIME AND GROUP

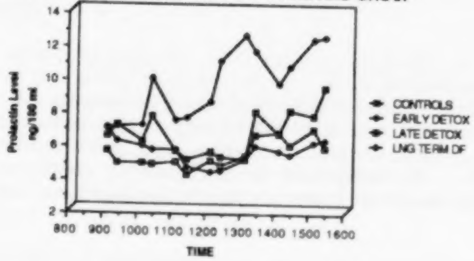


TABLE 3B. TESTOSTERONE LEVELS BY TIME AND GROUP

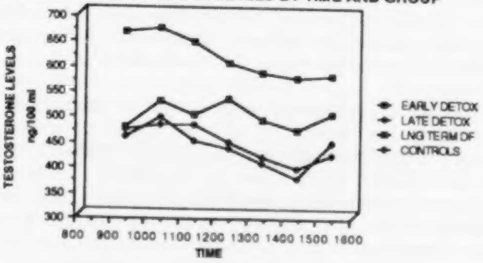
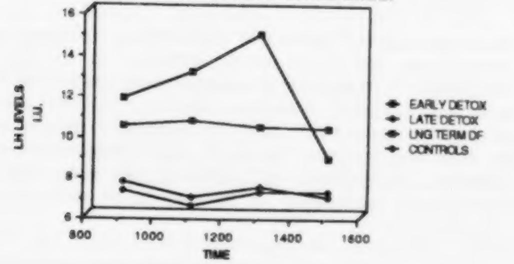


TABLE 4B. LH LEVELS BY TIME AND GROUP



The Reduction of Intravenous Heroin Use, Non-Opiate Abuse and Crime During Methadone Maintenance Treatment: Further Findings

J. Ball, E. Corty, H. Bond, C. Myers and A. Tommasello

ABSTRACT

As part of a three state study of the effectiveness of methadone maintenance treatment, data from 617 addict patients were obtained with respect to their lifetime and in-treatment characteristics. The Addiction Severity Index (ASI) was employed along with supplementary items on previous treatment and criminal behavior.

It was found that there was a marked reduction in intravenous heroin use after admission to treatment and a lesser reduction in the use of non-opiate drugs. Significantly, the overall incidence of drug abuse continued to decline with time in treatment; thus, the long-stay cohort (more than 4.5 years) had the lowest incidence of abuse while in treatment.

The reduction in criminality after admission to methadone maintenance treatment was dramatic; all 14 types of crime declined markedly - most over 80 percent. The reduction in criminality was associated with time in treatment. Thus, mean crime-days per year at risk decreased as follows: (1) during addiction period when "on the street" = 306.8; (2) in treatment .5 to 4.5 years = 24.0; (3) in treatment more than 4.5 years = 18.0 crime days per year.

INTRODUCTION - RESEARCH ISSUES ADDRESSED

Contemporary research has established that there is a marked reduction in heroin use and crime among addicts in the United States after admission to methadone maintenance (MM) treatment. This finding has far-reaching ramifications as it indicates that a nationwide modality of treatment can successfully impact those addicts whom it reaches. Thus, the fact that there is a notable reduction in intravenous heroin use and criminal behavior among the 72,000 out-patients currently in methadone maintenance programs is significant.

Although there is general agreement that methadone maintenance treatment reduces both heroin use and crime, a number of further research questions remain to be addressed:

1. To what extent does non-opiate abuse and alcohol abuse persist (or increase) after admission to methadone clinics?

2. What amount and types of crime persist after admission to methadone maintenance treatment? How is such crime affected by long-term stay?

3. How soon after admission does heroin use fall off? Does it cease with long-term treatment?

RESEARCH METHOD

The present study is part of a federally supported project investigating the effectiveness of methadone maintenance programs. New admissions and patients already in treatment at six MM programs were interviewed concerning addiction related problems by 12 professional, extramural interviewers. The interview schedule consisted of the Addiction Severity Index and supplementary questions on treatment experiences and criminal behavior.

SUBJECTS

Male patients who were admitted to these six programs during an eight week period in the fall of 1985 were eligible for participation. In addition, at each of these programs a random sample of male patients who had been in treatment for six months or longer was drawn and interviewed during the same eight week period (these patients were interviewed again in 1986).

PRETREATMENT CHARACTERISTICS OF 617 MALE HEROIN ADDICTS

The 617 male patients selected for study were in six methadone maintenance programs in three cities: New York, Philadelphia and Baltimore. The mean age of the 617 male patients at time of first interview (in late 1985) was 36.3 years. With respect to race and minority group status, 51.2 percent of the patients were black, 40.7 percent white, 6.3 percent Hispanic and the remaining 1.8 percent were Orientals, American Indians and of other races. Thus, 59.3 percent of the patients were minority group members.

From their onset of opiate use as teenagers, almost all the subjects used heroin for many years. Thus, their mean years of heroin use was 11.1 years and this was supplemented by use of other opiates as well. In this latter regard, 30.5 percent were regular users of other opiates and these had 8.0 mean years of such use.

In addition to their history of opiate addiction, these patients also abused an assortment of non-opiate drugs. The two most common were marihuana and cocaine, each of which was regularly abused by over half of the addicts (72.9 & 59.7 percent respectively). In both these cases, the drugs were consistently used over several years (10.4 years for marihuana and 5.7 years for cocaine).

Next in lifetime prevalence were "other sedatives" (34.0 percent), amphetamines (24.5 percent) and barbiturates (24.1 percent). These three classes of drugs were used for some 2.9 years to 5.4 years each. Hallucinogens and inhalants were the least frequently abused drugs. Hallucinogens were regularly abused in the past by 17.7 percent of the addicts; inhalants by only 6.7 percent.

Use of alcohol to intoxication on a regular basis was quite common within this addict population. Thus, 50.7 percent reported one or more years of such abuse and the mean years of regular use to intoxication for these was 8.4.

Most of the 617 male methadone maintenance patients had a considerable history of prior treatments for drug abuse. Indeed, 94.3 percent reported such prior treatment and the mean number of episodes was 4.0. With regard to prior methadone maintenance treatment, 28.0 percent have never had methadone maintenance treatment before; 29.3 percent have been in methadone treatment once before; 31.0 had 2 or 3 prior treatments, 11.7 percent have had 4 or more prior methadone treatments. In addition to prior treatment for drug abuse, some 11.9 percent of these patients have had prior treatment for alcohol abuse.

CRIMINAL HISTORY AND INCARCERATION

Most of the male methadone maintenance patients in the present study have a long history of criminal behavior, arrests and incarceration. Thus, the 617 males reported 4,681 prior arrests at time of interview. (or a mean of 8.8 arrests per person for the 532 who had been arrested). With respect to type of arrest, the following percentage of the 532 males have been arrested for these offenses:

1. Shoplifting - 36.5%
2. Parole violation - 34.8%
3. Forgery - 14.5%
4. Weapons offenses - 33.5%
5. Burglary - 43.6%
6. Robbery -27.1%
7. Assault - 28.6%
8. Arson - 2.1%
9. Rape - 1.7%
10. Homicide - 6.2%
11. Other non-drug offense - 14.3%
12. Drug business - 71.1%

In addition to arrests, the addict patients reported on their usual day-to-day offenses prior to current treatment. These data indicate the actual extent of criminality while arrests do not since less than one percent of crime-days result in arrest. With regard to day-to-day criminal behavior during their last addiction period, they reported that they engaged in crime some six days a week. That is, 54.3 percent reported daily crime, 22.4 percent engaged in crime 1 to 6 times per week and 21.4 percent reported no crime on a regular weekly basis.

As might be expected, the incarceration history of these addicts is extensive. Prior to their present treatment, 65.6 percent of the addicts had been incarcerated and their mean years behind bars was 3.8 years. Significantly, 7.3 percent had 10 or more years of incarceration before this treatment.

REDUCTION IN DRUG ABUSE AND CRIME DURING METHADONE MAINTENANCE TREATMENT

The 617 addict patients who were interviewed in late 1985 at six methadone maintenance programs were a stratified representative sample of the 2,394 patients in these clinics. Thus, there were 126 new admissions, 342 average stay patients and 149 long-term patients.

The extent of drug abuse among these 617 male patients in methadone maintenance treatment is tabulated in Table 1. As expected, the extent of opiate use among the admission sample was high, with 66.1 percent reporting heroin use in the past 30 days and 14.5 percent reporting use of other opiates. Inasmuch as some of those patients came from jail or other incarceration, this sample was in transition from various stages of their addiction status to stabilization on methadone. With respect to non-opiate use, this sample - or at least a major portion of it - was simultaneously involved in the abuse of these drugs. Thus, 58.0 percent abused cocaine, 48.8 percent used marihuana, 38.7 percent used alcohol to intoxication and 31.5 percent used sedatives other than barbiturates. In sum, these new admissions were significantly involved in non-opiate drugs prior to or during admission (Table 1).

Those patients who were in treatment for .5 to 4.5 years reported markedly lower incidence of drug abuse. With respect to the use of heroin, 23.4 percent reported continued use, but such use was only for 6.3 days per month. Use of other opiates similarly declined from 14.5 percent (of admission sample) to 8.3 percent of the moderate-stay sample. For non-opiate drug use, there also was a general decline in the percent who used these drugs. There was not, however, an appreciable decline in marihuana use (48.8 vs. 47.0 percent) and the overall frequency of use per month for those who did abuse non-opiate drugs did not change.

The long-term impact of methadone maintenance is evident in the extent of drug abuse among those in treatment for more than 4.5 years. Among these patients, both opiate use and non-opiate use has almost ceased (with the exception of cannabis and alcohol abuse). Thus, 92.4 percent report no use of heroin and 96.6 percent report no use of other opiates. For non-opiate drugs, 95 percent or more report no use of barbiturates or amphetamines; 83 percent were not using cocaine. Somewhat higher rates persist for marihuana use (37.9 percent) and alcohol use to intoxication (30.3 percent).

REDUCTION IN CRIMINAL BEHAVIOR AFTER ADMISSION TO TREATMENT

In order to obtain baseline information on patients' criminality prior to treatment, patients were asked to recount the types and frequency of offenses committed during their last addiction period.

A marked reduction in crime occurs after patients are integrated into methadone maintenance treatment. This reduction in criminality is evident with respect to all types of crime. Indeed, the general uniformity of the rate of reduction of criminality for 14 types of crime is striking. That is, the fact that 10 of the 14 types of crime decline by 80 percent or more is an unexpected finding; more variation and less decline were anticipated.

The extent to which crime was reduced during treatment can be seen from a comparison with the crime rates for the moderate length of stay and long-term stay patients.

Mean Crime Days Per Year:

Pretreatment:	When Addicted,	(N=600)	306.8
Intreatment:	Moderate Stay,	N=345	24.0
Intreatment:	Long-Term Stay,	N=145	18.0

CONCURRENT REDUCTION IN HEROIN USE AND CRIME DURING TREATMENT

The concurrent reduction in heroin use and crime following admission to methadone maintenance treatment is tabulated in Table 2. For the new admission sample, many of the addicts had already given up heroin or crime or both by time of interview. But this sample was in transition during the 30 day period of record, so cautious interpretation of these findings is indicated.

Most of the addict patients had completely given up both heroin use and crime after 6 or more months in treatment. Thus, 66 percent of the patients in the moderate stay sample reported no use of heroin and no crime. Significantly, most of those who were not complete successes (i.e., heroin days or crime-days in past 30) engaged in one or the other, but not both. But of the 23 percent who reported some heroin use, the mean days per month of use was only 1.4. Comparable findings with respect to crime were that 19 percent continued with some offenses, but again the mean days were (comparatively) low - only 2 crime days per month.

By the time patients had been in treatment over 4.5 years, both heroin use and crime had almost ceased! Thus, 83 percent of the long-stay sample were complete successes; 13 percent reported either some heroin use or crime, but only 4 percent reported both. But again, the frequency of heroin use or crime was low -0.9 days per month for those who used heroin and 1.5 days per month for those still involved in crime.

CONCLUSION

Investigation of the lifetime and current characteristics of 617 patients in methadone maintenance treatment reveals that there was a marked reduction in both opiate and non-opiate drug abuse during treatment. Concomitantly, there was a notable decline in criminal behavior.

It was found that length of stay in treatment was significantly related to this reduction in drug abuse and crime; heroin use and crime continued to decrease after one or more years in treatment. These findings support the efficacy of long-term methadone maintenance treatment.

At the same time, a number of collateral issues still need to be addressed. Are there model programs in terms of effectiveness? For example, which combinations of the five major components (methadone, counseling, urinalysis, medical and administration) of methadone maintenance treatment are most effective? What is the impact of treatment upon rehabilitation and psychiatric status?

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Table 1. Frequency of Opiate and Non-Opiate Drug Use in Past 30 Days For Three Treatment Cohorts: New Admissions, Moderate Stay and Long-Term Methadone Maintenance Patients.

	Admission (N = 124)		Intreatment .5 to 4.5 years (N = 338)		Intreatment Over 4.5 years (N = 145)	
Drug Type	Pct.	Mean Days	Pct.	Mean Days	Pct.	Mean Days
Heroin	66.1	19.0	23.4	6.3	7.6	11.1
Other Opiates	14.5	11.3	8.3	7.5	3.4	15.0
Barbiturates	4.0	9.0	1.8	5.7	2.8	15.0
Other Sedatives	31.5	8.3	21.6	11.5	14.5	14.7
Cocaine	58.0	13.5	26.6	8.8	17.2	6.3
Amphetamines	5.6	2.9	3.6	4.1	2.1	11.0
Cannabis	48.8	15.1	47.0	16.6	37.9	14.1
Hallucinogens	0.0	0.0	0.3	0.0	0.0	0.0
Inhalants	0.0	0.0	0.0	0.0	0.0	0.0
Alcohol (intoxication)	38.7	11.7	32.5	10.8	30.3	7.7

Chi square tests for each drug type used are significant at the .01 level, except for cannabis which was not significant.

(missing data on 10 cases).

Table 2. The Reduction of Heroin Use and Crime By Time in Methadone Maintenance Treatment For 617 Male Patients.

Status Past 30 Days	Last Addiction Period (N=617) Percent	New Admission Sample (N=126) Percent	Intreatment .5 to 4.5 years (N=342) Percent	Intreatment Over 4.5 years (N=149) Percent
• Both Heroin and Crime	81.0	29.4	7.6	4.0
• Only Heroin Use	19.0	37.3	15.8	3.4
• Only Crime Days	----	7.9	11.1	9.4
• Success	----	25.4	65.5	83.2
TOTAL:	100.0%	100.0	100.0	100.0

NOTE: Success was defined as no heroin use days or crime days in past 30 days.

The Chi square value for this table is 303.8 which is significant at the .01 level.

A 2.5 Year Follow-Up of Abstinence and Relapse to Cocaine Abuse in Opioid Addicts

T. Kosten, B. Rounsaville and H. Kleber

ABSTRACT

During a 2.5 year follow-up of opioid addicts, we examined psychosocial antecedents and consequences of the onset and remission of cocaine abuse. Patients whose cocaine use increased during follow-up had more severe problems than either those whose use decreased or those who never used cocaine. Furthermore, the attainment of cocaine abstinence among abusers was associated with improved psychosocial functioning, while the onset of cocaine abuse was associated with increased problem severity. Compared to drug-free and detoxification alone treatments, methadone maintenance may minimize legal complications of cocaine abuse, but otherwise it did not significantly reduce psychosocial morbidity from increasing cocaine abuse.

INTRODUCTION

Cocaine abuse has become a significant problem among opioid addicts, with the rate more than tripling from 1974 to 1978 (1-3). In previous reports, we examined the rate of cocaine abuse among methadone maintained patients in 1978-80 and in 1983 (2,3). In 1978-80 cocaine abuse was very common among opioid addicts, but methadone maintenance treatment appeared to be associated with reduced cocaine use (2). However, in a follow-up conducted in 1983, patients on methadone maintenance were reporting more cocaine use than those opioid addicts not in any active treatment (3). In the present longitudinal analyses we have focused on several dimensions of treatment outcome that go beyond drug abuse among the cocaine abusing opioid addicts. The central question is whether increased cocaine abuse during the follow-up was associated with deterioration in other dimensions of these patients' functioning or was cocaine use a more circumscribed problem for these patients. Early studies emphasized the benign nature of cocaine use, but more recent work has suggested that cocaine abuse may have adverse consequences (4,5).

To facilitate the analysis of antecedents and consequences of cocaine use, our 2.5 year follow-up will be divided into 6 month blocks and the three six months before, during and after the two index events (abstinence or onset of use) will be compared. If psychosocial problems "cause" cocaine abuse rather than being consequences of this abuse, then these problems should be severe for the six months preceding the onset of use and significantly lower during the six months preceding the attainment of abstinence. With the opposite findings (e.g. problems follow onset of use and precede attainment of abstinence), the conclusion that cocaine abuse leads to these psychosocial problems would be more likely. Finally, if attainment of abstinence or onset of use are unrelated to psychosocial problems, this will suggest that cocaine abuse may be a more circumscribed problem without widespread effects on psychosocial functioning.

METHODS

Three hundred and sixty-one (361) opioid addicts from the Substance Abuse Treatment Unit (SATU) of the Connecticut Mental Health Center were targeted for follow-up evaluation 2.5 years after the initial evaluation (3). From this target sample 268 were relocated and agreed to participate. The followed-up subjects included 48% whites, 76% males, 27% currently married, 47% unemployed. These subjects had a mean age of 27.6 ± 4.8 years and had used opiates for 9.3 ± 4.2 years. Eight subjects were not included in these analyses because of incomplete data on cocaine use.

Drug abuse and treatment participation were elicited using forms based on the Client Oriented Data Acquisition Program (CODAP). The frequency of heaviest abuse during the previous 6 months for each of ten classes of drugs was rated using these 7 point CODAP scales. Outcome was assessed using the Addiction Severity Index (ASI) at follow-up interview and 100 point scales assessing outcome during the five 6-month blocks of this 2.5 year follow-up. These 100 point scales covered criminal activity, social adjustment, employment, physical disability, global outcome, and opiate, cocaine and alcohol use.

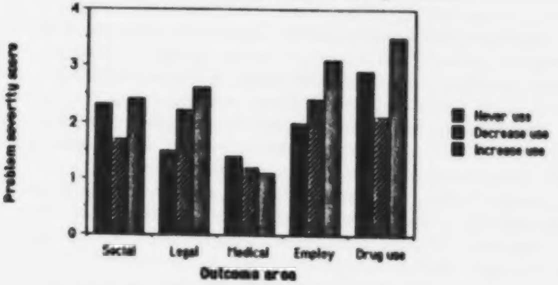
RESULTS

In the top panel of the Figure are shown the scores on the six ASI scales for three cocaine use categories: never used (None), decreased (Less) use, and increased (More) use of cocaine during the follow-up. In general, patients with increased cocaine abuse had more severe problems in the areas of drug abuse, legal problems, employment, and family/social problems. Psychological and medical problems showed no significant differences among cocaine use groups. A two way analysis of variance (ANOVA) was also performed for each outcome factor using treatment type (methadone, drug free or detoxification alone) and change in cocaine use as main effects. None of the interaction terms across cocaine abuse and treatment type were statistically significant. However, there were three significant differences among the treatment types. Significant differences occurred among the treatment types for the drug abuse, family/social and psychological problem areas. When covariance adjustments were made using ASI scores at intake, however, the three treatments significantly differed only in legal problems with the methadone patients showing the least legal problems on the ASI.

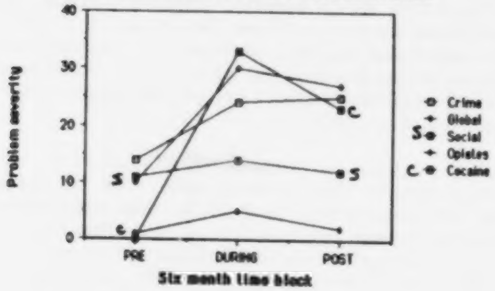
To assess the time period surrounding attainment of abstinence and the onset of cocaine use, the 30 months of the follow-up were divided into 6 month blocks. An episode of abstinence was defined as 6 months with no cocaine use. Fifty-two cocaine abusing patients attained abstinence for at least 6 months during the follow-up. Forty-four patients had the onset of cocaine abuse during the follow-up, and 36 of them had not been using cocaine when they initially applied for treatment. The other 8 patients had been using cocaine at entrance to treatment, but had then become abstinent and relapsed to cocaine abuse after being abstinent for at least 6 months.

The antecedents and consequences of starting and stopping cocaine abuse were examined by comparing the ratings made for the 6 months before (Pre), during (During) and after (Post) the index events of starting or stopping cocaine abuse. The middle panel of the Figure shows the problem severity scores for the onset of cocaine use. When patients began using cocaine (During time block), they developed significantly more severe problems with criminal activity, global assessment of functioning, social adjustment with family, and opioid use. These problems continued to be severe for the subsequent six months (Post time block), in spite of a drop in cocaine use severity for the Post

Cocaine use & Addiction Severity Index



Onset of cocaine use & clinical status



Cocaine abstinence & clinical status

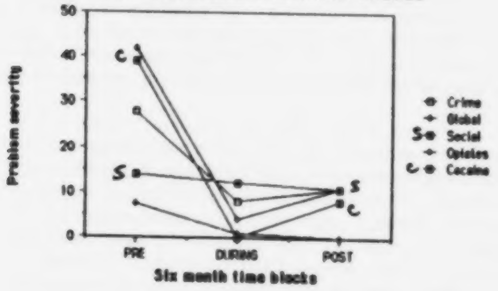


Figure 1

6 month period. Three other areas (employment, physical disability and alcohol use) showed no significant differences between any two time periods. The relationship of attaining cocaine abstinence to clinical status is shown in the bottom panel of the Figure. It shows that with cocaine abstinence (During time block), problems of criminal activity, global assessment, family social adjustment, and opioid use all decreased significantly. Overall, problems in each of these areas substantially decreased when the patient became abstinent from cocaine and increased when he relapsed to cocaine use.

DISCUSSION

During this 2.5 year follow-up of opioid addicts we found that those who increased their frequency of cocaine abuse (e.g. from monthly to weekly or from weekly to daily cocaine use) had more severe problems on a wide range of outcome dimensions. Psychosocial problems were generally more severe among the cocaine users whose use increased than among those whose use decreased or who had never used cocaine. Furthermore, social adjustment deteriorated substantially with the onset of cocaine use and improved substantially with attainment of abstinence, as shown by the 6 month blocks analysis. In general, psychosocial complications of cocaine abuse did not differ among the three treatment types, although the risk of legal problems appeared to be significantly less for cocaine abusers in methadone maintenance. This may simply reflect the more frequent illegal activities associated with non-methadone patients' seeking of illicit opioids along with cocaine.

Two factors may be important in leading abusers to abstain or at least decrease their cocaine use - medical problems and legal pressure. Medical problems were significantly worse among those whose cocaine use decreased rather than increased. This suggested that medical complications of cocaine use such as hepatitis or other infections for I.V. users may have contributed to these patients decreasing their cocaine use. The legal system may also apply external pressure on cocaine abusers. This speculation about legal pressures leading to reduced cocaine use is consistent with the higher levels of criminal activity preceding attainment of abstinence that we found and with patient reports that their cocaine "runs" would often be interrupted by an arrest and brief incarceration. Since legal and medical problems may motivate cocaine abusers to decrease their abuse, treatment programs should be prepared to address these problem areas at admission to treatment. Early medical interventions in treatment might include careful medical evaluations on admission with appropriate laboratory studies. Early legal interventions might include provision of legal aid counseling to insure that addicts get treatment rather than simply incarceration.

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Treating Cocaine Abusing Methadone Maintenance Patients with Desipramine

T. Kosten, F. Gawin and B. Schumann

ABSTRACT

Intravenous cocaine use in methadone patients has become a major problem because of both the complications of cocaine use and the potential spread of AIDS. During an eight week open trial we found that desipramine reduced cocaine craving and use in eight cocaine abusing methadone maintenance patients compared to eight patients getting no medication execept methadone.

INTRODUCTION

Cocaine abuse has increased over the last ten years becoming a major problem within methadone maintenance programs (MMP) with up to 40% of MMP patients reporting cocaine abuse (1-3). Because about 80% of the cocaine abusers in our MMP use it intravenously, contracting and spreading acquired immunodeficiency syndrome (AIDS) by IV cocaine use is also a risk. During a 2.5 year follow-up of opioid addicts that was completed in 1983, we found that the high rate of cocaine use in our MMP had minimally declined, despite extensive opioid oriented treatment (3). Furthermore, severe (more than weekly) cocaine abuse appeared to have increased. Thus, treatment interventions are needed not only to reduce the morbidity associated with cocaine itself, but also to reduce the spread of AIDS and HIV infection among MMP patients. Pilot work with desipramine by our group has demonstrated substantial reductions in cocaine craving and use (4). Thus, desipramine appeared to be a good candidate for further study among cocaine abusing MMP patients.

METHODS

Sixteen methadone maintained patients who had been abusing cocaine for at least three months at a level of over 1.5 grams per week were offered desipramine in addiction to weekly group counseling in an educational cocaine group. Eight patients chose to start on desipramine and eight decided to not use it. The patients included six females, 12 whites and had a mean age of

35.4 years (range = 29-47 years). The desiprmaine and unmedicated groups did not significantly differ in any of these characteristics. The despiramine group included four females, six whites and had a mean age of 33.4 (+2.5) years. The unmedicated group included two females, six whites and had a mean age of 37.5 (+5.5) years.

All patients were administered the medication daily under supervision at the clinic to insure adequate doses were being taken, and six of eight patients on desipramine had blood levels after at least 10 days on a stable dose. The mean desipramine dose was 141 mg daily (range = 75-200 mg) with a mean blood level of 219 (+84 s.d.) ng/ml. All patients remained on a stable dose of methadone with a mean level of 58 mg daily (range = 25-80 mg).

Patients' cocaine use and craving were recorded weekly for eight weeks and then followed for about six months after first starting treatment. Cocaine craving was assessed using a 20 point analog scale, and cocaine use was assessed by self-reported number of grams. Because no administrative sanctions were imposed for cocaine use during the first eight weeks in the cocaine group, we felt that self-reports of use during his eight week open trail were reliable, although cocaine use was also monitored by random weekly urine toxicologies.

RESULTS

Weekly craving scores were calculated as percent of craving at entrance into the trial (baseline). Both groups showed decreases in craving for the initial eight weeks of the trial and at the six month follow-up. Statistical analysis of the data for the eight weeks using repeated measures ANOVA demonstrated a significant time effect ($F=9.4$, $P<0.01$), showing that cocaine craving significantly decreased over time for both groups. However, neither the treatment main effect ($F=1.2$. $P<0.3$) nor interaction effect ($F=1.6$, $P<0.2$) were significant. However, using a response defined as a 50% or greater reduction in cocaine craving, 25% of the unmedicated had this response at week eight compared to 75% of the desipramine patients (Fishers exact test, $P=0.03$). Thus, desipramine appeared to significantly reduce cocaine craving relative to no medication. Similar analysis of the endpoint or "follow-up" data yielded a significant difference in craving reduction (50% unmedicated vs 100% desipramine, Fishers =0.04).

Reduction in cocaine use was consistent and sustained for the desipramine group, but very uneven for the unmedicated patients. A sudden drop in cocaine use at week four for the unmedicated group may have reflected a threat of administration sanctions at eight weeks, if cocaine use was not reduced. Urine results support this dramatic reduction in use, but craving was not substantially reduced and cocaine use returned. The desipramine group reported more extensive cocaine use (5.6 grams vs. 2.8 grams) for the month before starting in the cocaine abuse treatment, but for the week before starting both groups were equivalent (2.1 vs. 2.2 grams). Amount of cocaine use declined significantly during treatment using a repeated measures ANOVA (F=2.7, P<0.05). At week eight, desipramine patients were not statistically significantly more often abstinent (25% vs. 63%-desipramine, Fishers = 0.14), but the trend clearly suggested that desipramine decreased cocaine use among MMP patients. Urine toxicologies were also obtained weekly on a random schedule. These urine results also demonstrated an effect of desipramine on cocaine use. The unmedicated group had an average of 1.04 (+ 0.87) cocaine positive urines, while the desipramine group had an average of 0.22 (+ 0.22) cocaine urines (t =5.4, P<0.001).

DISCUSSION

Although preliminary findings from several trials have suggested the efficacy of desipramine in cocaine abusers not in a MMP, there are five major reasons why efficacy needs to be assessed in a controlled trial with cocaine abusers in the special situation of an MMP.

1. In this study, the difference in urines positive for cocaine use was statistically significant, and cocaine craving substantially and significantly decreased suggesting that replication in a larger, randomized trial is warranted. Moreover, because this study involved a small sample with no random assignment of patients to treatment groups, its conclusions are tentative.

2. The client characteristics of cocaine abusers in a MMP may be different from "pure" cocaine abusers. In previous studies, our group has shown that MMP cocaine abusers tend to be antisocial and significantly more often black males (2,3). Other disorders, including major affective and anxiety disorders, have been more prevalent among the cocaine abusing opioid addicts than among non-abusers (2,3). These findings imply that cocaine abuse treatment, particularly within methadone programs, may need to differ substantially from treatments for "pure" cocaine abusers. The high rate of antisocial personality disorder suggests that treatment needs to be structured with administrative sanctions as part of treatment. The greater than expected rates of affective and anxiety disorders suggest that the use of antidepressant medications may be doubly indicated for many cocaine abusing MMP patients. An important component of future studies will be to examine whether these comorbid psychiatric disorder have remained prevalent among cocaine abusing MMP patients, and whether these comorbid disorders have prognostic significance in cocaine abuse treatment.

3. The pattern and subjective effect of cocaine abuse may be different in MMP populations. For example, abuse may be more severe and escalate more quickly to larger amounts of use, and it is more likely to be by the I.V. route of administration. The "speedball" abuser may be quite different from other cocaine abusers. Methadone may also potentiate the euphoric effects of cocaine by limiting cocaine induced anxiety.

4. The interactions of desipramine with methadone maintenance may be important. In this preliminary study, a possible interaction appeared, since quite low doses of desipramine produced adequate blood levels and side effects were tolerable. Other drug interactions including interactions with cocaine may differ between MMP patients and non-MMP cocaine abusers.

5. There have been no studies describing how to integrate cocaine abuse treatment into the ongoing structure of a MMP. Although patients who select methadone maintenance may be particularly appropriate for pharmacological adjuncts to cocaine abuse treatment, these medications need to be provided in a psychotherapeutic context. We have developed an interpersonal therapy approach to cocaine abuse, and future studies might combine this type of therapy with pharmacotherapy for cocaine abusers in an MMP. This psychotherapy focuses on interpersonal cues for cocaine use.

In summary, cocaine abuse in MMPs is common, dangerous (particularly with AIDS in I.V. drug use), and not well treated using current methods. In fact, current MMP methods may aggravate the problem and persistence of cocaine abuse. To find new treatments for it within MMPs, we have looked to pharmacological approaches that we have developed with non-MMP patients. We have examined desipramine as the most potentially useful and have found that it reduces both cocaine craving and use. This initial pilot trial indicates that a more expensive controlled clinical trial with desipramine among MMP patients seems promising and worthwhile.

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Counselor Differences in Methadone Treatment

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INTRODUCTION

This report results from an unusual occurrence within the methadone maintenance program: the resignation of two experienced counselors within one week. Both counselors had worked in the program for a minimum of five years and neither counselor was released for disciplinary reasons. One counselor had to resign because of an accident; the other resigned because illness in the family required relocation. Since both counselors had caseloads of forty active patients; and since the resignations occurred so abruptly and within such a short period of time, the patients were essentially randomly assigned to other counselors over the next three-day period.

ROLE OF COUNSELING

Because the clinic collects weekly record of urinalysis results, prescriptions for psychotropic medications, methadone dose, arrest and employment rates, it was possible to compare the performance of each patient during the six months prior to his transfer with the six months following reassignment to the new counselor. We reasoned that this unusual situation would provide a relatively pure test of the contribution of counseling without the artificiality of an experimental setting and without the influence of the multiple factors generally associated with assignment or reassignment of patients in the normal clinical situation.

ROLE OF THE COUNSELOR

There is a large and still growing literature devoted to evaluating the effectiveness of different "types" of counselors. In particular professional counselors (ie. those with masters level education) para-professional counselors (ie. those with less than a masters level education) and ex-addict counselors (ie. those with prior histories of substance abuse who may or may not have a masters level education) have been compared in more than fifty studies. These groups of counselors have been compared on a variety of patient performance measures including during and post-treatment drug use (Aiken _et al._, 1984a; 1984b), client retention (Brown & Brewster, 1973; Brown *et al*., 1985), and overall client adjustment following treatment (Brown *et al*., 1985; LoSciuto *et al*., 1984). The clear finding from these studies is that *as a group*, there are no significant differences in the performance of patients assigned to these "types" of counselors' and that "...**paraprofessionals achieve clinical outcomes equal to or significantly better than those obtained by professionals"** (Durlak, 1979, p. 80 empahsis added).

The finding of no significant *group* differences among these "types" of counselor has led to the suggestion that there may be no **individual** differences among counselors generally. In this regard we felt the data from this study were similar to data from our earlier study of two types of professional psychotherapy for opiate addicts (Woody et al., 1983; 1987). In that study, we too found no significant *group* differences between two conceptually and methodologically different therapies, each practiced by trained therapists. However, subsequent examination of data within each therapy group revealed major outcome differences among individual therapists (Luborsky et al., 1982; 1985; 1986). Thus, in the work to be reported here, we had the opportunity to make individual comparisons of counselors with regard to the benefits they had provided to their methadone maintained patients.

METHOD

Subjects - were 61 opiate-dependent, male veterans in methadone maintenance treatment at the Philadelphia VA Medical Center. These individuals were a subgroup patients who had been assigned to the two counselors in that program who resigned unexpectedly and abruptly within a week of each other in early 1985.

In order to standardize the patient sample and to provide comparable and representative measurement periods, only patients who had been in treatment at least **six months prior** to transfer and who stayed in treatment **six months following** reassignment were included for study. Four counselors received 12 or more transferred patient (N's = 12, 16, 16, 17) and these caseloads were considered adequate for study.

Patient Reassignment - As a result of the large number of patients affected by the counselor resignations and the abruptness of the situation, the normal clinic policy of contacting individual patients and counselors to develop mutually satisfactory reassignment "matches" *was not followed*. Instead, the senior clinical supervisor was forced to reassign the affected patients in a rapid manner using *essentially* random assignment procedures. Assignments were made within three days and were based entirely upon the size of the remaining counselors' caseloads.

As a test of the results of the reassignment procedure on caseload mix we performed comparisons of the demographic and background characteristics of the patients who were assigned to the four caseloads. Measures included age, race, number of prior treatments, duration of time in the methadone treatment program, years and months they had been assigned to the transferring counselor, number of different counselors they had been assigned previously, years of alcohol use, years of opiate, stimulant and depressant drug use, number of criminal convictions, methadone dose and prescription of an ancillary psychotropic medication (e.g. for sleep, anxiety or depression problems). Finally, the four caseloads were compared with regard to the proportion of patients that were transferred from each of the two resigning counselors.

All comparisons showed non-significant differences and the greatest difference found was only $p<.14$. The results of these comparisons as well as the nature and rapidity of the assignment procedure convinced us that there were not explicit or overt differences among the caseloads at the time of transfer and that the reassignment procedure had produced essentially random distributions to the four counselors.

Standard Program Measures - All patients in treatment at the Philadelphia VA Drug Dependence Treatment Center are required to provide a supervised urine specimen weekly on a randomized schedule. Also available are weekly records of each patient's methadone dose and records of any ancillary psychotropic medications prescribed by the program physicians. In addition, monthly reports indicated whether the patient had been employed and/or arrested during the reporting month. Because all of these measures have been standard program requirements it was possible to compare them among counselors (See below), over the 12 months of observation (six pre and six post-reassignment).

RESULTS

Five measures were considered in the evaluation of patient performance for each counselor's caseload over the six month periods prior to and following the transfer point: the percentage of the caseload that had been employed or arrested, the proportion of patients with positive urine results, the proportion that was prescribed ancillary psychotropic medications (eg. for problems with sleep, anxiety or depression) and the average methadone dose of the caseload.

PRE-TRANSFER DIFFERENCES

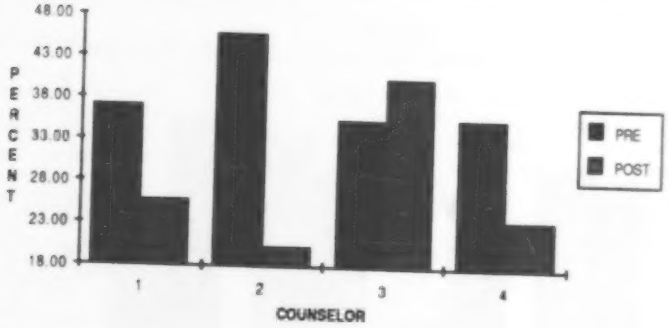
One way (between-counselor) repeated measures (across months) analyses of variance were performed on all five of the performance measures for the period prior to transfer. Results of these analyses revealed no significant between-counselor differences ($p>.10$), no differences across the six month pre-transfer period ($p>.10$) and no counselor-by-months interactions effect ($p>.10$) on any of the measures examined.

Post-Transfer Differences

Given the general tendency for comparable and stable performance measures in the caseloads prior to the transfer, we questioned whether there were improvements after the transfer point and if these improvements were different among counselors. We therefore performed a treatment (counselor) by levels (pre to post transfer), repeated measures (month) multiple analysis of variance (MANOVA) on all of the five patient performance measures. Results indicated significant differences between levels ($p<.01$) and between ($p<.03$) and therefore permitted a more detailed examination of the individual measures using two way (counselor by transfer) repeated measures ANOVAs. The results are discussed individually below for each measure.

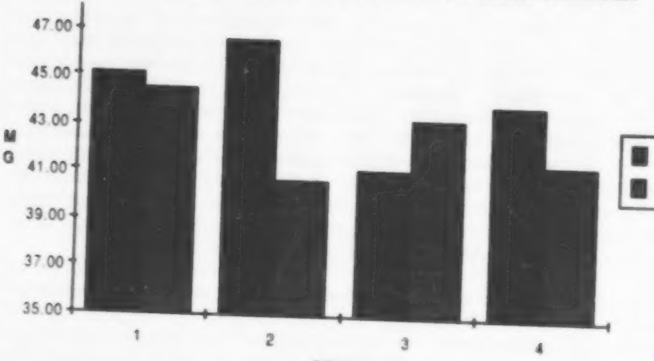
Urinalysis (Fig. 1) - A urine was considered positive if results showed **any of the following:** opiate, cocaine, amphetamine, barbiturate, benzodiazepine; or if methadone was absent from the specimen. As can be seen, there was a significant counselor-by-transfer interaction in the analysis of variance ($F=4.89$, df3,40, $p<.01$).Subsequent paired comparisons among the counselors on the post-transfer urine results indicated that counselor 3 differed significantly from all other counselors ($p<.05$ or less) who did not differ from each other)$p>.10$).

FIGURE 1
POSITIVE URINES BEFORE AND AFTER COUNSELOR TRANSFER



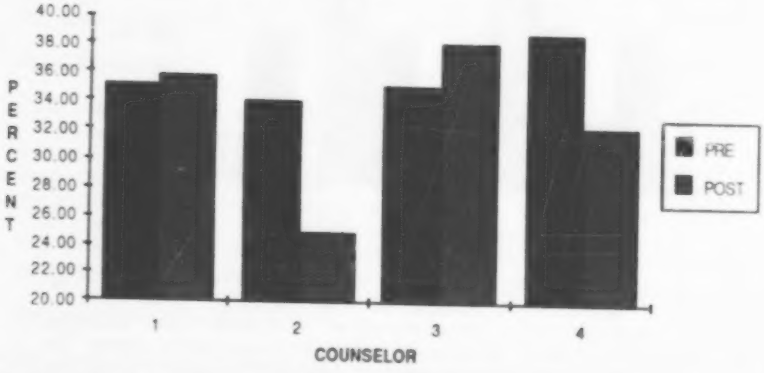
Methadone Dose (Fig. 2) - Results of the analysis of variance indicated a significant effect of the transfer ($F=8.16$,df1,40,$p<.01$) and for the interaction of counselor and transfer ($F=8.23$,df3,40,$p<.01$). Subsequent paired comparisons among the caseloads during the post-transfer period indicated that the caseload of counselor 2 had a significantly lower average methadone dosage than the other caseloads ($p<.05$), which did not differ from each other ($p<.10$).

FIGURE 2
METHADONE DOSE BEFORE AND AFTER COUNSELOR TRANSFER



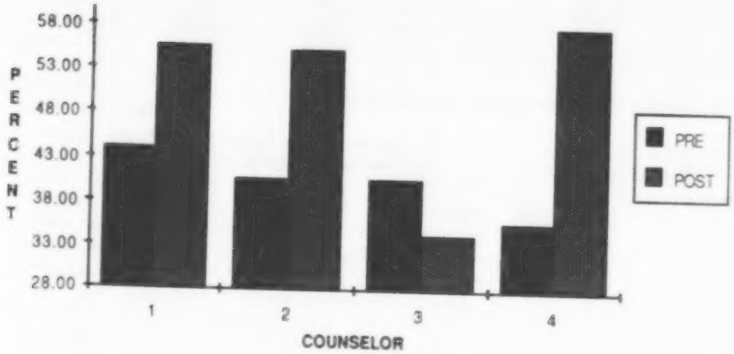
Ancillary Psychotropic Medications (Fig. 3) - Although there was no general change in the proportion of patients receiving medication across the transfer point (p>.10), there were significant differences in the nature and amount of change among the caseloads (Interaction F=3.71,df3,40,p<.05). For example, counselor 3 showed a modest increase (p<.10) in the proportion of his/her patients receiving prescribed medication, while counselor 2 actually showed a significant decrease (p<.05).

FIGURE 3
% ON MEDICATION BEFORE AND AFTER COUNSELOR TRANSFER



Employment (Fig. 4) - As in the case of the urine results there was a significant overall improvement following transfer (F=49.21,df1,40,p<.01). Again, a comparison of the caseloads revealed that the patients of counselor 3 had a siginificantly lower employment rate (p<.05) than the other caseloads, which did not differ from each other (p>.10).

FIGURE 4
% EMPLOYED BEFORE AND AFTER COUNSELOR TRANSFER



Arrests (No Figure) - The proportion of patients arrested at any time was generally low (mean=3.7%) throughout the period of study. Thus, neither the pre to post-transfer changes nor the post-transfer differences among the counselors were statistically significantly (p>.10).

DISCUSSION

When the performance of the caseloads was examined during the six month periods prior to and following the transfer point across five objective measures of patient status (methadone dose, urine screening, prescription of ancillary psychotropic medications, employment, and arrest rates) there were marked and consistent differences in outcome among the four counselors. One counselor (#2) significantly reduced illicit drug use and unemployment in the caseload while concurrently reducing the average methadone dose and the proportion of patients prescribed ancillary medications. In contrast, another counselor (#3) showed **worsened** status in employment, drug use, and criminality in the caseload while concurrently **increasing** the average methadone dose and the proportion of patients prescribed psychotropic medications. The remaining two counselors (#1, #4) showed improvements in employment, drug use and arrest rates in their caseloads while maintaining approximately the same average dose of methadone and the same proportion of patients receiving psychotropic medications.

It should be noted that we have seen differences of this nature and magnitude among professional psychotherapists treating randomly assigned caseloads of methadone-maintained opiate addicts in another study (Luborsky et al., 1985; Woody et al., 1983). In that study, one therapist showed an average improvement rate of more than 100% across seven outcome measures in 14 randomly assigned patients. Another psychotherapist with comparable training and experience showed an average change rate of -4% across the same measures and a similar randomly assigned subject population (See Woody et al., 1983 for details).

What accounts for Counselor Differences?

With regard to prior treatment experience however, there were few differences among the four counselors with all of them averaging more than eight years in the field. The possibility that formal education differences were implicated is more difficult to evaluate. Counselor 2 had a masters degree in psychology and had received a state license to practice. Counselor 3 was a recovering addict with a high school degree. These differences alone suggest that formal education has a profound effect. However, when the backgrounds of the other counselors are considered, the conclusion is much less clear. For example, one of the resigning counselors also had a masters degree in psychology, while three of the other counselors (1,4 and one of the resigning counselors) had bachelors degrees in psychology. Thus, as has been reported in a large number of prior studies (See Durlak, 1979 for a review), the differences seen among the caseloads of these counselors cannot easily be attributed to educational differences.

As a mean of examining differences in the actual process of counseling we turned to the patient charts to get some indication of the rehabilitation strategies of the individuals involved in the present study. There were definite and consistent differences in the written work among the counselors studied. For example, with regard to the written notes of counselors 1 and 4, the charted information suggests that they performed their counseling duties in a concerned and organized manner. The charts from these counselors showed thorough and accurate charting of all pertinent aspects of patient contact. These two counselors had a clearly formulated plan of rehabilitation that had been worked out through consultation with the treatment team and the patient. Further, the notes from these charts suggested that the initial plans were generally followed and documented at all points during the rehabilitation process. The majority of the patients in these two caseloads were seen more than once per month, with many seen weekly. The day-to-day problems of their patients were dealt with through referral to program resources such as physicians, nurse, employment counselor, letters to legal authorities and public assistance agencies as well as encouragement, support and basic, sensible advice.

In contrast, the information from the charts of counselor 3 and the two counselors who resigned, indicated far less organization in the treatment plans and far less detail in the treatment notes. There was little indication of consistency in enforcement of program rules (e.g., take-home regulations, loitering, etc.) and very little documentation of the use of program resources for their caseloads (e.g., employment counselor, nurse, etc.). Perhaps most striking was the general lack of documentation surrounding changes in methadone dose and prescription of ancillary medications. Finally, the indications were that the patients in these caseloads were seen less frequently than the patients of the other counselors (average slightly more than once per month).

The data from the charts of counselor 2 indicated the same high level of organization and consistency as was exhibited by counselors 1 and 4. However, the nature of the notes during rehabilitation process suggested a different content in the sessions and in the counseling process. The approach indicated in the notes of this counselor was one anticipating problems in the patient and discussing strategies to deal with the anticipated situations, thereby focussing the rehabilitation on the development of new behaviors and new ways of thinking by the patient. In this regard it should be noted that this individual now has a private therapy practice.

A Comment Regarding Counseling and Counselors

The performance differences seen among these counselors are all the more interesting in that there is now a large and widely quoted body of controlled studies that have shown no significant performance differences among groups of counselors divided on the basis of formal education or past history of addiction (See Durlak, 1079; Aiken et al., 1982 for reviews. This body of work is important to the field of addiction rehabilitation in that the distinction between "ex-addict" or "recovering" versus "professional" counselors has generally represented significant ideological differences in treatment strategies. Since this group distinction has had such ideological significance the finding of no group differences in performance has been seen by some as justification for the hiring of predominantly "ex-addict" or "recovering" counselors since these counselors can generally be hired for less money than counselors with higher levels of education and since "the literature shows that there are no differences in performance". Still others have used these findings as an indication that counseling is a generic commodity of relatively little importance in the overall rehabilitation process.

It therefore becomes extremely important to note that this well-replicated finding (as well as the data presented here) means only that addiction background and formal educational level, are by themselves, **not well related to therapeutic performance;** and therefore are neither good nor sufficient criteria to use in hiring a counselor. By illustration, the finding that there were no performance differences between left and right handed counselors would not be justification for hiring predominantly left-handed counselors, "since there were no differences in performance". These findings **explicitly do not mean** that there are **no** indications of therapeutic performance, or particularly, that there are **no individual** distinctions among counselors. We do not pretend to know the important pre-employment personal, ideological and experiential qualities that are related to therapeutic efficacy in counselors or therapists. At this point, the data suggest that the initial performance of the counselor, as judged by his treatment plans, written notes and the objective indications of improvement in his/her caseload, may be the best indication of future therapeutic efficacy.

In summary, given the presented data with all the noted cautions and qualifiers, we feel two conclusions are possible. First, counseling is an "active ingredient" in the rehabilitation of drug-dependent patients even in a treatment modality where medical, and pharmacological interventions are also prominent. Second, as seen in earlier studies of psychotherapists (Luborsky *et al.*, 1985; 1986), there are significant differences in effectiveness among counselors. Some promote rapid and sustained change in their caseloads and others actually detract from the effectiveness of the other components of treatment. It will be important in future studies to examine the personal and procedural qualities associated with effective counseling and therapy and then to work to implement the findings as a means of improving the general efficacy of substance abuse treatment.

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Occurrence of Central Nervous System Defects in Fetal Alcohol Syndrome

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Alcohol consumption during pregnancy has been documented to cause multiorgan anomalies and developmental retardation. Experiments in animals exposed to alcohol in early pregnancy demonstrate a significant incidence of central nervous system defects including absence of the corpus callosum. The frequency of this and other CNS anomalies in neonates with FAS is unknown. To determine the incidence of CNS anomalies in FAS we studied all neonates admitted with the diagnosis of FAS admitted to our nurseries by CT Scan or neurosonography.

Thirty-seven neonates (22 males, 15 females), with a mean gestational age of 35 weeks (range 29-34 weeks) and mean birth weight of 1680 gms (range 539-3345) were examined. Abnormalities were detected in 7 patients. Four patients had evidence of absence of corpus callosum, two had evidence of prominent cortical atrophy, and another had mild ventricular dilation. The 4 patients with absence of the corpus callosum had strong phenotypic expressions of FAS.

The 19% incidence of detectable CNS anomalies in FAS suggests that CT and/or neurosonogram should be considered in evaluation of infants suspected of this syndrome. The correlation of these findings with neurologic and developmental abnormalities will need to be determined by long term followup studies.

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Prenatal Alcohol Exposure, Cognitive and Motor Development in the First Year

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The goals of this study were to determine: (1) whether differences in the developmental outcomes of children prenatally exposed to alcohol can be correlated with the amount of alcohol consumed by the mother during pregnancy; (2) whether there are specific areas of cognitive, motor and/or behavioral development in the first year which are more vulnerable to the effects of alcohol; (3) whether the pattern of observed deficits in affected children varies with the duration (the length of exposure during gestation) of maternal alcohol use.

The sample was selected from the population of women receiving prenatal care at Grady Memorial Hospital, a large inner city hospital in Atlanta, Georgia. Three groups of women were recruited for study: (1) women who reported alcohol use equivalent to one ounce or more of absolute alcohol per week throughout pregnancy; (2) women who drank an equivalent amount, but discontinued alcohol use in the second trimester of pregnancy; (3) women who did not drink at all during pregnancy. Follow up psychological testing was done on the infants of all women participating in the study at six and twelve months of age using the Bayley Scales of Infant Development.

The results of this study indicated that: (1) discontinuing alcohol use during the second trimester of pregnancy was associated with better developmental outcomes as measured by the Bayley Scales of Infant Development; (2) behavioral differences were not significant at six months, but children exposed to alcohol throughout gestation performed significantly poorer than the other two groups at twelve months; (3) cognitive and language skills were more affected than motor development and social skills as measured by the Kent State scoring system for the Bayley; (4) a significant interaction was found between volume and duration of prenatal exposure for scores on the social skills subscale at one year.

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Children Exposed to Methadone *In-Utero:* Cognitive Ability in Preschool Years

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The outcome of infants born to drug dependent women maintained on methadone during pregnancy has been an area of special concern. The consequences of such exposure for the neonate and young infant have been widely investigated. However, there have been few studies to determine if preschool children exposed to methadone in-utero have impaired cognitive functioning. The purpose of this study was to evaluate the cognitive function of preschool children born to women maintained on methadone during pregnancy. Forty-five children (27 methadone exposed children (M) and 18 non-drug exposed comparison (C) children) were evaluated with the McCarthy Scale of Children's Abilities when the children were between 3 1/2 and 4 1/2 years of age. All children were participants in a longitudinal study examining developmental outcome from birth through 5 years of age. The mean daily maternal methadone dose during pregnancy was 38.42 mg and 92% of the children required pharmacotherapy for neonatal abstinence. Student t-tests revealed no difference between groups on the McCarthy General Cognitive Index (GCI) or any of the 6 subscales. Mean scores were: GCI(M) 106.51, (C) 106.05; Verbal(M) 53.44; Perceptual (M) 55.51 (C) 53; Quantitative(M) 51.33, (C) 53.38; Memory (M) 49.51, (C) 52.27; and Motor (M) 52.29, (C) 50.44. These results indicate that the general cognitive ability of children in the pre-school years who have been exposed to methadone in-utero is not impaired.

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A Retrospective Search for the Etiology of Drug Abuse: A Background Comparison of a Drug-Addicted Population of Women and a Control Group of Non-Addicted Women

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INTRODUCTION

For many years, researchers labeled substance abuse etiology as the consequence of an addictive personality. Researchers sought to discover the characteristics and the variable that distinguished addict and non-addict populations on a myriad of demographic and personality variables. "Instead of discovering just one reliable characteristic, they discovered at least eleven, none of which accounts for the majority of variance between groups, but each of which increases the probability of drug abuse to a certain extent." (Bry 1983, p. 255-256).

Therefore, the "one variable quest" was replaced by a search for "one" combination of the established variables that best explained substance abuse. Nearly every study, utilizing well-designed, multi-variate, and longitudinal analyses, produced a different set of explanatory variables, all of which are related to substance abuse in a statistically significant manner. However, not one explains more that a moderate amount of variance. While some commonalities exist, there are also discrepancies. For example, some researchers found relationships to parents among the important variables. Other studies measured the parental variable, but found it unimportant (Bry 1983).

Bry _et al_., (1982) responded to the variations by using the "multiple risk factors hypothesis." This is a notion that disparate findings are due to the fact that substance abuse is a function of the "number" of etiological factors instead of a particular set of them. "When this hypothesis was tested on a data set containing six of the eleven most commonly supported etiological characteristics, the results revealed a highly significant relationship between number of these characteristics and the probability of current abuse." (Bry 1983, p. 256)

Additionally, there was not one predominant combination of risk factors. Among the 156 subjects who exhibited two to four risk factors, twenty-four different combinations occurred. Not one of the combinations accounted for more than twenty percent of the cases (Bry 1983).

This research project was designed in order to target and to examine risk factors that are considered significant in the etiology of drug abuse. The project is a retrospective search which investigates and compares the backgrounds of a population of drug-addicted women and a non-addicted population of women. Additionally, the study examines the "numbers" of responses, and the "similarities" in the responses of individual users to particular etiological variables.

SUBJECTS AND METHODOLOGY

Twenty-eight drug-addicted women were asked to participate in this study, as they walked into the lobby of Family Center of the Thomas Jefferson University Hospital, Philadelphia, Pennsylvania. This occurred over a ten day, non-consecutive period. Family Center is a comprehensive outpatient methadone maintenance treatment program for drug and/or alcohol dependent women. The program recognizes the special needs of the pregnant addict, and provides patients and their children with medical, psychiatric, and social services. Twenty-four addicted women agreed to the interviews. Nineteen of the women were in the clinic to receive methadone for opiate addiction, and five came in to receive counseling for cocaine abuse. All five cocaine abusers were black. Eight of the opiate addicted women were black and eleven were white. All twenty-four were receiving one or more of the following assistance programs: Welfare; Medical Assistance; Women Infants and Children Services; and/or Maternal Infant Care.

Eight of the women were pregnant. Additionally, eight had delivered within the last year. Five of the women had delivered more than a year ago and were still receiving methadone and counseling from Family Center. Three subjects had special classifications. One had just aborted, and two subjects had miscarried.

A control group not affiliated with Family Center, and without drug or alcohol problems, was obtained in the following manner. Thirty subjects were approached as they walked into the Thomas Jefferson University Hospital Obstetrical and Gynecological Clinic (JOGA) for treatment regarding pregnancy, gynecological services, or family planning information. Additional requests for interviews were made to several JOGA women who had just delivered and were in the hospital. A total of twenty-two women agreed to be interviewed. Of the twenty-two, two were not appropriate, as they did not pass a screening questionnaire concerning drug and alcohol consumption (Vaillant 1977).

The "control" failure criteria in this research project for the screening questionnaire was more than five drinks per week and two affirmative answers on any of the other questions. In the drug use circumstance, "trying marijuana a few times" was passable if the subject was not presently smoking, and as long as there were not two affirmative answers on the other questions. If other drugs were used more than one time, the subject was considered to exhibit high-risk behavior, and was deemed inappropriate.

Subsequently, twenty non-addicted women were interviewed. Sixteen of those were black and four were white. Four of the subjects were interviewed postpartum. Six women were pregnant when interviewed, and six had delivered in the last year. The remaining four women were using either the JOGA gynecological services or the family planning services when interviewed. The controls met the same governmental assistance criteria as the drug-addicted subjects.

All subjects were administered verbally (by the author) the following questionnaires: 1) A Vaillant (1979) untitled questionnaire which indicates alcoholism in a family member or a friend. This questionnaire was modified in order to include drug use consideration. If three affirmative answers were received, the subject was regarded as having experienced alcohol and/or drug addiction in a family member; 2) Moos' Family Environment Scale (Moos 1974); and 3) A comprehensive social data questionnaire compiled from the social data forms prescribed by Family Center and the Hospital of the University of Pennsylvania. The drug-addicted women were given an additional questionnaire involving their drug history.

The subjects were reminded before each appropriate questionnaire that the answers should be given from a retrospective point of view - "How it was when you were growing up - not how it is now." The questionnaires were placed in the past tense where necessary.

The Family Environment Scale was of special interest in that the language utilized was not always understandable to the subjects, or certain words were interpreted differently than intended by the FES. For example, the phrase which states, "We rarely (hardly ever) went to lectures, plays, or concerts." The answer invariably would be the following (for blacks and whites): "Oh, yes we did, we went to lots of rock concerts." Because that was the explicit interpretation for those subjects, the answer was marked "false." This meant that they "did go to lectures, plays, and concerts."

Several words from the FES had to be defined for the subjects: "rarely," "spontaneous," "cultural," and "inflexible." The phrase stating, "We believed there were just some things you had to take on faith," had to be explained to twelve of the forty-four subjects. The point of these idiosyncrasies should be noted by anyone who does not administer the FES verbally.

The questionnaires sought variables which indicated either demographic information or the subject's perception of familial functioning; activities in childhood or drinking and/or drug patterns of family members. Analysis of the data consisted of determining the means and the standard deviations of the user group and the non-user group on all variables where applicable.

The results for each independent variable on the Family Environment Scale were compared using a Student's t-test. All data was measured for an "at or below" p = .05 level of significance. If any factor neared that level, it will be reported. A Chi-square was used for the mean age at first drug use by race.

The means of both groups were compared with the scores in Appendix A of the FES Manual. A percentage was obtained from the mean score. This percentage was compared with the FES "normal range" expectation of 50% to 60%. Ranges will be reported where significant.

Additional student's t-tests were utilized on a population consisting of all drug-addicted and all non-drug-addicted subjects who had experienced family or origin alcohol or drug abuse. The control population of drug-addicted and non-addicted subjects had not been exposed to familial alcohol or drug abuse. Probabilities were computed for each FES variable at or below the p = .05 level of significance. Specific and significant trends between the groups will be discussed.

RESULTS AND CONCLUSIONS

The mean age of the Family Center drug-addicted women was 30.6 years with a range of 23 to 41 years. The mean age of the non-addicted controls was 25.1 years, with an age range of 18 to 29 years. The age mismatch is indicative of the populations of Family Center and of JOGA. Where age difference is an issue, the discrepancies will be discussed.

The racial composition of the Family Center women consisted of 54% black and 46% white; the racial composition of the controls was 80% black and 20% white. The disproportionate number of blacks in the control population disallows racial comparisons between user and non-user groups on most variables. However, a comparison was made between blacks and whites in the drug-addicted population for the mean age at first drug use by race. While the Chi-square probability was not significant (p = .10), the percentages between the groups were significant. Seventy-three percent of the whites used drugs initially between the ages of 12 and 16. This is in contrast to 46% for the blacks. The mean age of the total user group for initial drug use was 15.7 years.

Seventy-nine percent of the Family Center group in this study are opiate addicted, and 21% are cocaine addicted. The drugs reported by the subjects to be first used included marijuana, heroin, amphetamines, valium, alcohol, barbiturates, tuinal, percodin, dilaudid, and quaaludes, in decreasing order. First drug use by 54% of the women was marijuana. Thirty-three percent of that group used marijuana in combination with one or more of the other drugs. Twelve and one-half percent of the women commenced with heroin only, and 8% with alcohol only.

Sixty-seven percent of the Family Center group experienced alcoholism of one or both parents. This is in contrast to 35% of the controls. Of that 35%, presently 57% of the controls have a drug and/or alcohol addicted sibling. Additionally, 8% of the Family Center women had one drug-addicted parent. Another 8% had a sibling at least one decade older with an alcohol abuse problem. Therefore, 83% of the drug-addicted women were from chemically dependent families.

Much has been said about the negative effects of single-parent households on the functioning of children. However, in this study an interesting dichotomy occurs. Sixty-five percent of the controls were from single parent households, while only 38% of the Family Center women lived with a single parent. Race may be a factor in the results, particularly since 80% of the controls are black. The high incidence of female-headed households in black lower socioeconomic groups is well researched. In the Family Center group, the racial composition of those living in single-parent households is 56% black and 44% white. The results may be affected by another factor as well. A study by Kumpfer and DeMarsh (in press) found that although abusing families were significantly less cohesive on both the FES and the Olson _et al._, FACES-II standardized tests, the marital dyads were significantly more cohesive or enmeshed than other randomly selected families. That particular kind of enmeshment seems to support the status of chemically-dependent families. Because 83% of the Family Center women come from chemically dependent families, the Kumpfer and DeMarsh research suggests a possible explanation for the higher incidence of dual-parent families in the drug-addicted women. An examination of the controls who were from chemically-dependent families reveals that 28% were from dual-parented white families where addiction occurred in one or both parents; 57% came from black female-headed households where addiction occurred in every female head; and 15% came from female-headed black households where the addicted father had left. In order to evaluate any significant links, the parental-dyads would have to be administered the FES and the FACE-II tests. As in most retrospective studies, the task would be enormous if not impossible. It does suggest, however, that the examination of the parental-dyads of adolescents who are in treatment would be valuable.

The educational and work histories of the Family Center group and the controls were notable. Over 50% of the drug-addicted women left school before the end of 11th grade. This is in contrast to 25% of the controls. Fifty percent of the Family Center women and 40% of the controls named Math and/or Science as their favorite subjects. Seventy-one percent of the addicted subjects and 80% of the controls received some kind of vocational or educational training beyond school. As indicative of drug-addicted individuals, Family Center women do not have substantial work histories. Twenty-one percent have not worked since 1978, only 42% worked at some time in 1986-87, and 8% have never been employed. Eighty-five percent of the controls have worked sometime in 1986-87.

In this research project, the issue of mean number of pregnancies between groups and the actual number of children is exacerbated by age and by drug use as well. Results for Family Center women and for the control women are as follows: Mean user gravida = 4.1; Mean user para = 2.2; Mean control gravida = 2.5; Mean control para = 1.6. Sixty percent of the controls had normal pregnancies in contrast to 38% of the addicted women. Thirty-four percent of the drug population had complications due to miscarriage and stillbirth, probably caused by drug use. Thirty-four percent of the Family Center women and 25% of the controls had abortions.

Both groups were asked how they perceived their childhoods, and they were asked to rate their feelings in the following way: "My childhood was: 1 - very happy; 2 - happy; 3 - neither happy nor unhappy; 4 - unhappy; 5 - very unhappy." Forty-five percent of the controls perceived their childhoods as "very happy," while only 12.5% of Family Center women answered similarly. When the responses to choices 3 through 5 were combined, 54% of the addicted subjects fell into that combination. Only 15% of the controls rated their childhoods in the same manner.

The groups were asked, "When you were growing up, did you wish you were someone else?" With the exception of one subject, all of the drug-addicted women answered "yes." By contrast, 30% of the controls answered affirmatively. The drug-addicted women were always specific about who or what they wished to be. For example, five black Family Center women had wished to be white, and one white woman had wished to be black. These results are far and above the usual and expected adolescent disgruntlement concerning identity.

Sexual and physical assaults are significant variables occurring in the drug-addicted women. Sixty-seven percent had been sexually assaulted (penetrated) in comparison to 15% of the controls. Of the drug-addicted sexual assault victims, 75% were raped under the age of 16 years, and the youngest had been 8 years. The same number of youth rapes occurred in the white and in the black drug-addicted women. Two of the addicted subjects were raped by more than one person during one encounter. One woman had been raped at the age of 13 years by three men, and another had been raped by nine men at the age of 9 years. Twenty-five percent of the drug-addicted women were raped by a father, brother, or grandfather.

Literature concerning enmeshment issues in families of drug addicts is prevalent (Coleman 1980; Stanton and Todd 1982; Friesen 1983; Davis and Klagsbrun 1977). Two of the processes involved in the concept of differentiation are "age at initial leave taking" and "circumstances surrounding returns." The mean age of initial leaving for the addicted women was 19.2 years, and 19.3 years for the controls. Sixty three percent of the drug-addicted women returned home sometime after they left originally, as compared to 25% of the controls. Of those, 33% of the addicted women, and 20% of the controls have remained. While the "return factor" may be an important variable in relation to drug abuse, one would suspect that the frequency of returns may be a more significant variable to examine. (Not available from this data.) Finances may be another complicating factor with the total population of this study.

The Family Center women and the controls were administered the Moos Family Environment Scale. None of the variables between the groups was statistically significant at the .05 level. However, the variables "cohesion" and "expression" yielded p values of .07. When the means of the control population are compared with means from samples published in the FES manual, the control population falls within the ranges specified as normal, with the exception of "expression." The Family Center women did not score within the normal ranges on cohesion, expression, conflict, or control. For example, when means were converted to percentages (appendix A of the FES manual), the addicted women scored 34% and the controls 61% on cohesion. The normal range is considered to fall between 50% and 60%. On expression, the Family Center women scored 35% and the controls 47%. While the controls did not score within the normal range, the scores were in the correct direction. When comparing conflict, the Family Center women scored 66% on conflict, contrasted by 55% for the controls. This suggests that if both of the populations were increased (even if the means remained the same), the level of significance would be at or below the .05 level. In addition, using the FES, all of the women in the user group and the non-user group who were "children of chemically dependent families" were compared with the user and non-user groups who were not from chemically dependent families. The "children of chemically dependent families" scored significantly lower on cohesion ($p = <.05$). There were no significant differences on any other variables. Once again, the means were in the correct directions, and the non-user scores fell within the normal ranges.

DISCUSSION

Criticisms of retrospective studies range from "recall problems" to discrepancies surrounding "circumstantial subjectivity." Undoubtedly, this study is influenced by such recollective problems. However, in terms of drug abuse and etiological issues, the study does reveal "high agreement factor percentages" among the users on a "number" of variables. The drug-addicted women experienced the following significantly similar background risk factors: chemical abuse within the family of origin (83%); "early" (before 16 years) sexual abuse (55%); lack of cohesion and expression in their families (19 of the 24 users scored below the 50% normal range on cohesion, 14 of those women scored below 40%; 18 users scored below the normal range on expression, 14 of those scored below 40%); familial conflict and control issues (18 users scored above 60% on conflict, 13 of those scored 70% or above; 14 scored above 60% on conflict); unhappy childhoods (over one half); and a lack of ego center (96% wished they had been someone else). This project suggests that this population of women is a rich source for research and for underestanding the cultural and social implications associated with the etiology of drug abuse.

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The Detection of Heroin, Cocaine and Cannabinoid Metabolites in the Stools of Infants of Drug Dependent Mothers

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We have shown (Dev Pharm Ther 1:163, 1980) in morphine addicted fetal monkeys that the tissue concentration of morphine was highest in their intestines due to bile secretion or swallowed fetal urine. To test the corollary hypothesis that the stools from infants of drug dependent mothers (IDDM) will contain the drugs which the fetus has been exposed to, in utero, we collected stools (meconium) during the first 5 days from 10 IDDMs and 2 control infants and tested them for the metabolites of heroin (morphine), cocaine (benzoylecgonine), and cannabis (delta-9-tetrahydrocannabinol or THC), three commonly abused drugs. In addition, we addicted a pregnant Sprague-Dawley rat by the daily subcutaneous injections of morphine sulfate (10-20 mgs/kg bid) from the 8th to the 21st day of gestation. Soon after birth, the rat pups were sacrificed and their intestines were collected for morphine analysis. The drug metabolites were extracted in appropriate solvents and quantitated by radioimmunoassay. The control stools were used for background correction. **RESULTS:** Four of the 10 IDDM stools contained morphine (range = 0.24 - 22.7 μg/gm stool, mean = 5.42) and 3 had cocaine metabolites (range = 0.24 - 0.67 μg/gm stool, mean = 0.42) up to the 2nd day of sampling. Five of 5 stools tested contained delta-9-THC (range = 0.023 - 1.082 μg/gm stool, mean = 0.37) up to the 5th day sample. In the 9 rat pups, their intestinal contents collectively contained 1.65 μg of morphine or 0.18 μg morphine per pup. **SIGNIFICANCE:** This study shows that the stool (intestinal content) is a repository of the drugs which the fetus has been exposed to in utero and their detection may provide a unique insight into the drug exposure of the fetus throughout gestation. Likewise, since the complete evacuation of meconium occurs slowly, drug detection for diagnostic purposes in infants, is feasible even in late sampling.

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Variable Apnea in Term/Preterm Infants Born to Drug Dependent Mothers: A Prospective Study

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Recent studies have suggested that infants born to mothers with drug dependency may be at greater risk for infant apnea and/or SIDS. In order to evaluate this relationship, 54 infants were prospectively studied with thermistor-pneumocardiograms. Fifteen infants (28%) were premature, while 39 (72%) were full-term. Mean B.W. was 2.75 ± .65 S.D. kg, mean G.A. was 37.9 ± 3.3 wks. Seven of 15 premature infants (47%) had abnormal studies (central apnea-2, obstructive apnea-2, periodic breathing 10%-3), whereas, 7/39 term infants (18%) had abnormal studies (obstructive apnea-2, periodic breathing 5%-4, mixed apnea-1). Infants in nearly every case had mothers with multiple drug addictions. These results contrasted to control groups, which demonstrated a 70% incidence of abnormalities in 55 well premature infants (p=NS) and a 2.9% incidence in 34 normal term infants (p .025). Children with abnormal studies were monitored until normal pneumocardiograms were attained. Infants were monitored for a mean of 3.8 ± 1.2 S.D. mos. Three infants (21%) were treated with methylxanthines also because of severity of apnea. No infant required resuscitation during monitoring. One parent refused to comply with monitoring as prescribed. These results suggest that maternal drug dependency has little effect upon apnea incidence in premature infants, but increases apnea frequency in term infants. Apnea requiring resuscitation, however, appears to be infrequent in this population if diagnosed early and treated with methylxanthines and/or home monitoring.

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Maternal Use of Cocaine, Methadone, Heroin and Alcohol: Comparison of Neonatal Effects

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While the effects of maternal narcotic use during pregnancy have been well defined, the effects of cocaine have not been as clearly delineated. We prospectively evaluated 55 infants for neonatal signs and symptoms associated with drug use during pregnancy. Four groups were identified (cocaine alone, cocaine and alcohol; cocaine and other drugs; and methadone and/or heroin). Cocaine was the most commonly abused drug (87%), far exceeding methadone abuse (42%, $p = 0.00008$). Need for treatment (Lipsitz > 6), duration of treatment, elevation of BP and thrombocytosis were significantly greater in methadone and/or heroin users compared to cocaine alone or in combination. Whether cocaine symptomatology is inherently less severe, or whether drug abuse among mothers who use cocaine differs from those who do not, remains to be elucidated.

	Cocaine	Cocaine + Alcohol	Cocaine + Drugs	Methadone + Heroin
No. Patients	20	12	16	7
m̄ birthweight (gms)	2728	2648	2373	2997
G.A. (wks)	38.4	37.2	36.2	39*
Lipsitz > 6 (# pts)	2+	3*	5*	6+*
Duration Rx (m̄ days)	9.5*	8.3*	15.6	31.0*
Peak B.P. (mmHg)	68.6/34.8*	71/37	80/41*	75/41
m̄ Platelets x103	345+	332*	401	544+*

(Cocaine compared to methadone alone + heroin) $p < 0.05$*; $p < 0.01$+)

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Cocaine Use During Pregnancy: Adverse Perinatal Outcome

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We report the neonatal outcome of 70 pregnancies complicated by cocaine use. Forty-two of the women used alcohol or marijuana in addition to the cocaine, and 45 women (65%) used cocaine throughout the pregnancy. Drug-free pregnancies were matched for age, parity, tobacco use and medical complications. Polydrug (non-cocaine)-complicated pregnancies (N=70) were matched for these same parameters plus marijuana and alcohol use. A comparison of fetal outcomes revealed:

Group	GA	Del<37	BW (all)	BW (>37)	BW < 2500
Cocaine	37.0	24%	2853±698	3094±438	24%
Polydrug	39.0	4%	2932±600	3180±490	6%
Drug-free	39.0	3%	3382±551	3433±453	4%
P value	<.001*	<.001**	<.001*	< .001*	< .005**

*ANOVA **X^2analysis

The incidence of IUGR (birthweight Brenner's 10th percentile for gestational age) in the cocaine group was 19% (N=13) vs 4% (N=3) in the polydrug and 3% (N=2) in the controls (P<.005). Abruptio placentae occurred in 17% (N=12) of cocaine vs 1.4% (N=1) each of polydrug and control patients (P<.005). Two of the cocaine-exposed infants suffered perinatal cerebral infarctions, and 3 additional cocaine-exposed infants had seizures in the neonatal period. No control or polydrug infants suffered these complications. Four cocaine and one polydrug infant were positive for HIV antibody in the neonatal period. Maternal cocaine use places the infant at increased risk for complications in the neonatal period.

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The Use of Tricyclic Antidepressants in Methadone Maintained Pregnant Women and Infant Outcome

L. Green, S. Ehrlich and L. Finnegan

Over the past two decades there has been considerable controversy over the use of tricyclic antidepressants in pregnancy. In the early 1970's the tricyclics were suspected of being teratogenic. A possible correlation was reported between imipramine use during the first trimester of pregnancy and infants born with reduction deformities of the upper limbs.(1) Subsequent epidemiological reports on birth defects out of Atlanta and Los Angeles were unable to establish any connection between tricyclics and birth defects and the general conclusion was drawn that if tricyclics have teratogenic potential in humans it is of low order at therapeutic doses.(2) When Greenberg, *et. al.*, (1972) compared maternal drug use during pregnancy in a congenital abnormality and no abnormality group the use of tricyclics was similar in both sets of mothers.(3) Concurrently, in the early 70's, other neonatal effects were reported with tricyclic use. Shearer, *et al.*, (1972) associated the use of the tricyclic, nortriptylene in pregnancy with urinary retention in the neonate.(4) There have been reports of withdrawal symptoms in neonates born to mothers on imipramine and desipramine.(5,6) Animal studies have suggested more subtle teratogenic effects. Mason and Von Pellen (1978) reported increased adrenergic responses in the uteroplacental vasculature with prior administration of imipramine and amitriptylene in the pregnant ewe.(7) Coyle and Singer (1974) reported impaired maze performance in rats with *in utero* exposure to imipramine.(8) Coyle and Singer (1975) reported behavioral unresponsiveness and an absence of the characteristic histological changes associated with enriched rearing conditions when rats were exposed to prenatal imipramine.(9) Simkins, *et al.*, (1985) looked at the progeny of rats treated maternally with doxepin and imipramine and concluded that tricyclics produce subtle alterations in selective adrenergic function. Simkins also looked at trimester differences and found third trimester exposure with imipramine reduced birth weight and growth rate of progeny and found that first and second trimester doxepin and third trimester imipramine increased infant mortality.(10)

Owaki, *et al.*, (1971) found no teratogenic effects in rats exposed to *in utero* doxepin hydrochloride even at doses 400 times the recommended clinical doses(1). The literature is also filled with reports and letters from physicians of cases where tricyclic pregnencies produced offspring without harmful effects to the fetus.(12,13,14,15,15,17) Conclusive proof is, however, lacking and the controversy remains.

This preliminary study was undertaken to examine the data from 1983 to 1986 of infants born to mothers on methadone maintenance and whose clinical status warranted the use of tricyclics during pregnancy.

Methods:

The patients were enrolled in Family Center, an out-patient voluntary program under the auspices of Thomas Jefferson University Hospital designed to offer extensive medical care and psychosocial services for the pregnant drug-addicted woman and her infant. The dictum which has been adhered to at Family Center, was to prescribe tricyclics only in the second and third trimester of pregnancy and only if warranted by severe clinical depression.

To determine the outcome of infants born to mothers on tricyclic antidepressants, a population for comparison was selected which consisted of two groups. Group 1 consisted of 17 infants[1] born to women on methadone maintenance who had received imipramine (n=2) or doxepin (n=15) during various trimesters of their pregnancies: first and second trimesters (n=1), first, second and third (n=1), second and third (n=5) and third trimester only (n=10). All patients in group 1 were diagnosed as clinically depressed and prescribed tricyclics in low doses between 25 mg and 100 mg which were administered on a daily basis along with methadone maintenance. Low doses of tricyclics were considered due to pregnancy, the potential synergistic interaction between tricyclics and methadone, (18) and clinical results demonstrated at these doses. The two patients who received tricyclics during the first trimester became pregnant while on the medication. The group II patients consisted of 18 infants born to women randomly selected from the same pool of Family Center patients on methadone maintenance but not receiving tricyclics. The maternal variables compared between the groups were: age, race, gravidity, parity, methadone dose, number of prenatal visits and socioeconomic status. Illicit drug use information was obtained by self report and by routine urine toxicology and compared between the groups. The infant variables compared between groups were: gestational age, birth weight, head circumference, intrauterine growth, one and five minute Apgar scores, length of hospital stay and neonatal abstinence. A comparison analysis was then applied to control for in utero benzodiazepine drug exposure as benzodiazepine use in pregnancy and methadone maintenance is of concern.(19,20,21,22) Group comparisons were analyzed using a student t-test for differences between numerical mean measures or a chi-square (x^2) analysis for the frequency data. Statistical differences were measured at or below the p=.05 level of significance. Mean trimester differences between infant variables within the tricyclic group were then compared by gross analysis. Any infant complications were noted for comparison and evaluation.

Results:

A comparison of group I - tricyclic prescribed methadone maintained women and group II - non-tricyclic methadone maintained women revealed the following:

Maternal Demographic Characteristics (Table 1) - The ratio of Black to White women was 7/10 in group I and 10/8 in group 2. The average ages were 30.4 years and 29.8 years, respectively. The average number of prenatal visits was 5 in group 1 and 6.1 in group 2. Mean gravidity values were 5.2 and 4.6 and mean parity values were 2.9 and 1.8. This difference in parity was significant at the p=.05 level. The average methadone doses were 47.5 milligrams and 46.9 milligrams, respectively.

TABLE I
Demographic characteristics of 35 pregnant women

	Group I (n=17) (tricyclic/methadone)	Group II (n=18) (methadone)
Mean Age (years	30.4	29.8
Race # (%)		
Black	7 (41%)	10 (56%)
White	10 (59%)	8 (44%)
No. of Prenatal Visits	5	6.1
Mean Gravidity	5.2	4.6
Mean Parity	2.9	1.8*
Mean Methadone Dose (mg/day) (at delivery time)	47.5	46.9

*Significant at the p=.05 level.

Drug Use During Pregnancy (Table 2) - The illicit drug use during pregnancy revealed the use of illicit heroin, benzodiazepines, cocaine, other opiates[2], propoxyphene, glutethimide and alcohol in both groups. The illicit use of barbiturates and amphetamines and the prescribed use of barbiturates and amphetamines and the prescribed use of thioridazine were confined to the tricyclic group only.

TABLE II
Maternal drug use during pregnancy**

Drugs	Group I (tricyclic/methadone) n=17 (%)	Group II (methadone) n=18 (%)
Heroin	10 (59%)	17 (94%)
Benzodiazepines	13 (76%)	7 (39%)
Cocaine	4 (23%)	6 (33%)
Other Opiates***	4 (23%)	3 (17%)
Propoxyphene	3 (18%)	3 (17%)
Glutethimide	3 (18%)	1 (6%)
Barbiturate	2 (12%)	0
Amphetamines	1 (6%)	0
Thioridazine	1 (6%)	0
Alcohol	2 (11%)	1 (6%)

**Information obtained by self-report upon enrollment at Family Center Program and by routine urine toxicology report.
***Codeine, hydromorphone, morphine, oxycodone or non-identified opiate.

Outcome of the Neonate (Table 3) - The between group comparisons of neonatal outcome variables which included birthweight, head circumference and Apgars at one minute were lowest in group 1. None of these differences were found to be significant at the p=.05 level. Mean gestational age of group I infants was 37.3 weeks compared to 38.7 weeks for group II infants. This difference was significant at the p=.05 level.

TABLE III
Infant outcomes

	(Group I) (n=17) tricyclic/ methadone group	(Group II) (n=18) methadone group
Mean Birth Weight (gms)	2798.5	2996.4
Mean Gestational Age (wks)	37.3*	38.7
Mean Head Circumference (cm)	32.7	33.3
Mean Hospital Stay (days	15.9	19.5
Intrauterine growth (#)		
SGA	1 (6%)	
AGA	16 (94%)	18 (100%)
LGA		
Mean Apgar Scores		
1 minute	6.6	7.5
5 minute	8.3	8.3
NAS (Neonatal Abstinence Score)		
Treated (#)	10	11
Paregoric	6	8
Phenobarbital	4	2
Paregoric & Phenobarbital	-	1
Not Treated	7	7

*Significant at the p=.05 level.

Benzodiazepine as a factor in outcome of the neonate (Table 4) - When the benzodiazepines were controlled for and all subjects from the study were placed into a benzodiazepine group I, and a non-benzodiazepine group II, there were no significant differences between these groups for any of the infant parameters compared.

TABLE IV
Factorization of benzodiazepine use and infant outcome

	Group I methadone/ benzodiazepine exposed infants (n=20)	Group II methadone/non- benzodiazepine exposed infants (n=15)
Mean Birth Weight (gms)	2802.5	3030.7
Mean Gestational Age (wks)	37.6	38.6
Mean Head Circumference (cm)	32.8	33.3
Mean Apgar Scores		
1 minute	7.0	7.5
5 minute	8.3	8.8

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Outcome of the neonate - Trimester differences (Table 5) - Infant outcome variables were sub-divided for comparison between trimester tricyclic use. The tricyclic group was sub-divided into four groups: First and second trimester (group I), second and third trimester (group II), first, second, and third trimester (group III) and third trimester only (group IV). The comparison revealed that birth weight, gestational age, head circumference and Apgar scores at 1 and 5 minutes were lowest in group IV. These third trimester only mean infant variables were lower than total tricyclic group averages. Small numbers and differences in trimester distribution made data inappropriate for statistical analysis.

TABLE V

Trimester Tricyclic Use and Infant Outcome

	Sub Group I 1st & 2nd trimester only	Sub Group II 2nd & 3rd trimester	Sub Group III 1st, 2nd & 3rd trimester	Sub Group IV 3rd trimester only
# cases	n=1	n=1	n=5	n=10
Mean birth wt. (gms)	3895	3500	3022	2507
Mean gest. age (wks)	42	39	37.6	36.6
Mean head circum. (cm)	35.5	34	33.9	31.72
Mean Apgar scores				
1 minute	3	8	7.8	6.4
5 minute	9	9	8.6	8.1
Mean hosp. stay (dys)	23	7	11.8	18.2
NAS treated (#)	1	0	2	7
Phenobarbital	1	0	0	3
Paregoric	0	0	2	4
not treated (#)	0	1	3	3

Outcome of the neonate - Infant Complications (see Table 6) - There were 5 cases of infant complications and 1 SIDS death at age 1 month in group II. There were 8 cases of infant complications in group I. The complications in group I appeared more extensive in nature than controls. Some of the group I complications were identified with early gestational age.

TABLE VI
Infant Outcome - Infant Complications

Group I		Group 2	
PT. #1	meconium aspiration	PT. #1	candidiasis
PT. #2	asphyxia neonatum, sepsis, hyperbilirubinemia of prematurity	PT. #2	transient tachypnea
PT. #3	hyperbilirubinemia of prematurity, possible transient tachypnea of newborn	PT. #3	sepsis (no positive cultures)
PT. #4	mild meconium aspiration, pneumonia	PT. #4	post maturity
PT. #5	hypopyrexia, neonatal depression secondary to general anesthesia	PT. #5	conjunctivitis (staphylococcus)
PT. #6	hyperbilirubinemia of prematurity, sepsis	PT. #6	SIDS at 1 month
PT. #7	hyperbilirubinemia of prematurity, septicemia (clostridium butylicum) oral candidiasis klebsiella gastroenteritis		
PT. #8	palate deformity		

Discussion

This study suggests that the infants of mothers of methadone maintenance who are concurrently on low doses of tricyclic antidepressants are born somewhat earlier than infants born to methadone maintenance mothers not prescribed tricyclics. The findings of lower mean birth weight, smaller mean head circumference, lower mean Apgar scores and the increased severity of infant complications seen in the tricyclic exposed infants were not significant but may be attributed to earlier gestational age. Of additional interest is the finding that, when gross comparisons were made according to trimester prescribed, infants exposed during the third trimester only were born earlier and smaller than infants exposed in any other trimester combinations.

These results are presented, however, with some limitations. First the matching of demographic maternal characteristics cannot eliminate any inherent differences in the profiles of diagnosed and treated depressed patients as compared to presumably non-depressed patients. Differences in gravidity were determined attributable to the potential differences in patient profiles. Second, and perhaps related to the first, are differences in illicit drug use in the two groups. Although these differences were taken into account in the analysis, illicit drug use remains a general limitation in studying this population.

Depression remains a problem in pregnant drug dependent women.(23) The tricyclic, doxepin has been established as an effective treatment for the relief of depression and anxiety in patients on methadone maintenance.(24) Depression and anxiety may represent a stress to an already high risk pregnancy. Within the context of these considerations along with the findings drawn from the study, little more can be provided than to support what has previously been suggested: that tricyclics can only be prescribed to pregnant methadone maintenance patients when the potential benefits outweigh the carefully considered risks.

In conclusion, this study found that infants born to methadone maintained women exposed to *in utero* tricyclic antidepressants were born earlier than infants of methadone maintained women not exposed to tricyclics. It was also an observation that the more severe complications were seen in the tricyclic exposed infants and were attributable to the earlier gestational age.

Future investigation should attempt to collectively study greater numbers of infants born to women on tricyclics in both the drug addicted as well as the general population before tricyclic use in pregnancy can be unequivocally accepted or rejected.

Footnotes

1. Subject no. 18 of group I was eliminated when it was established that tricyclic use began subsequent to a diagnosis of fetal abnormality.
2. Other opiates included codeine, hydromorphone, morphine, oxycodone or non-identified opiate.

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REFERENCES AVAILABLE UPON REQUEST FROM AUTHOR

Pediatric Surveillance of AIDS Among Infants of I.V. Drug-Using Mothers

D. Kaplan, R. Kletter and J. Ferrara

POPULATION STUDIED: Infants of I.V. drug using mothers

SAMPLE SIZE: Twenty seropositives, 20 controls

TIME FRAME: Three years

METHODOLOGY

Weekly for first month; monthly for next six months; bi-monthly for following six months; quarterly for next 12 months. Complete history and physical, anthropomorphic measurements, immunizations, lab workups.

BASIC FINDINGS CONCERNING WOMEN AND/OR CHILDREN

Significant seropositivity rate in population of i.v. drug using mothers and their infants. Study data to present time does not indicate significant progression of disease up to two years of life.

CURRENT OR FUTURE RESEARCH STRATEGY

Following the experience of New York/New Jersey, increased numbers of infected children will require policy consideration for day care, foster care and school attendance. As number of seropositive women increase, potential for isolating predictive variables among i.v. drug using women may help to identify mothers and their infants at greatest risk for infection (e.g., chaotic lifestyle, uncontrolled drug use, unsafe sex practices, criminal involvement).

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Maternal Drug Abuse During Pregnancy and Pharmacotherapy for Neonatal Abstinence Syndrome

L. Finnegan

Newborns exposed in-utero to psychoactive drugs frequently undergo abstinence. This study evaluated: 1) the relationship between the type of maternal drug use and the incidence of neonatal abstinence and 2) which of 3 treatment drugs was most effective -- paregoric, phenobarbital, or diazepam. Abstinence was assessed by a scoring system related to drug dose. Successful treatment was considered when one drug controlled abstinence. Of the 300 infants, 59% were treated for abstinence and 41% required no treatment. Maternal drug use consisted of opiates, non-opiates and varying combinations of both. Infants exposed to non-opiates in-utero were less likely to undergo abstinence (36%) than those exposed to opiates (58%) or both (70%). The mean number of days to control symptoms of abstinence was 7.6 days, and duration of treatment averaged 38.6 days. If maternal drug use included opiates alone, paregoric was the drug most successful in controlling abstinence (87% of infants). In maternal non-opiate use, phenobarbital was most effective (100%). In maternal opiate and non-opiate use, paregoric was most effective (88%). Treating an infant with diazepam indicated the need for a second treatment drug in 70% of cases, regardless of maternal drug use. These data suggest that: 1) effective treatment for neonatal abstinence is related to the type of maternal drug use, 2) there is a higher incidence of abstinence in infants prenatally exposed to opiates alone or in combination with non-opiates, and 3) diazepam is generally ineffective for the treatment of neonatal abstinence.

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Separation of Opioid Receptor Blockade from Production of Functional Supersensitivity

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Chronic treatment with opioid antagonists increases brain opioid receptors (upregulation) and enhances the potency of opioid agonists (supersensitivity) such as morphine. In the present study we have examined the role that blockade of the analgesic action of the μ receptor ligand morphine and the δ receptor ligand [D-Ala^2-D-Leu^5]enkephalin (DADLE) plays in the production of supersensitivity. Mice were implanted subcutaneously with a high (7.5mg) or low (2.0mg) dose naltrexone (NTX) pellet or a placebo pellet for 8 days. Mice were tested for blockade of morphine (24mg/kg,sc) analgesia (tailflick) or DADLE (4.4nmol/mouse,intracerbroventricularly) at 4hr and 8 days following implantation of NTX. Whereas 95-100% of placebo-implanted mice showed complete analgesia 30min following morphine at both time points, both NTX doses blocked analgesia at 4hrs (100% of mice blocked) and 8 days (95-100% blocked). Similarly, DADLE (20min after administration) produced analgesia in 75% of placebo-treated mice at 4hr and 8 days following implantation, and both doses of NTX blocked DADLE analgesia at both time points (100% blocked). If blockade of analgesia is a sufficient condition to produce supersensitivity, then both NTX doses should also be equi-effective in increasing morphine's analgesic potency. Three groups of mice were implanted with the low or high dose of NTX, or placebo for 8 days. The pellets were then removed and 24hr later mice were injected with morphine (8mg/kg,sc) and assessed for analgesia. Consistent with our previous studies, this dose of morphine produced analgesia in 27.9% of placebo-treated mice. The same dose of morphine produced significant supersensitivity (70.7% analgesic) in high dose NTX-treated mice. The low dose NTX treatment did not produce a significant increase in analgesia (45% analgesic) compared to controls, and produced significantly less analgesia than the high dose NTX group. These results indicate that blockade of analgesia mediated by μ and δ receptors is not sufficient to produce a full supersensitivity response and raises the possibility that blockade requires only partial receptor occupancy and supersensitivity full receptor occupancy. Supported in part by NIDA grant # DA 04185.

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Colonic Opiate Receptors Change with Age: Preliminary Data

J. Culpeper-Morgan, P. Holt and M. J. Kreek

Elderly patients require lower doses of opiates for analgesia than do younger patients. We have reported that constipated elderly individuals increase stool wet weight and volume in response to oral and parenteral naloxone. Thus changes in the central as well as enteric human endogenous opioid system probably occur with age. We have started to measure the differences in the relative opiate subtype densities in the colons of young (6 weeks) and elderly (30-33 months) male albino Hartley strain guinea pig(GP)s. Crude membrane fractions of longitudinal muscle with attached myenteric plexus were prepared and suspended in 50mM Tris (pH 7.4 @ 25°C). Results of binding assays using 3H-naloxone displaced with naloxone and "universal agonist" etorphine, were analyzed by a least squares curve fitting routine (FITCOMP, PROPHET system). The table lists the relative nanomolar affinities (IC50) of each model, relative densities (%Bmax), and the significance of the improvement of fit of a 2-site versus a 1-site model.

		YOUNG COLON		OLD COLON	
		naloxone	etorphine	naloxone	etorphine
1-site	IC50	1780±870	82±20	250±160	84±40
2-sites	% Bmax-S1	-	-	43±27	50±25
	IC50-S1	-	-	20±50	1±2
	% Bmax-S2	-	-	57±20	50±7
	IC50-S2	-	-	5000±6500	1000±550
	p:	.431	.141	.028	.001

The 1-site model reveals an overall increase in affinity for naloxone with age and no change in etorphine affinity. The ability of FITCOMP to resolve the data from the elderly GPs into 2 sites of equal density whereas this is not possible with the data from the young animals implies a change in receptor subtype density with age. The increased affinity of mu preferring naloxone suggests an increase of mu subtype density. Full characterization of these differences requires analysis of competition experiments with highly selective ligands.

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U50,488H Differentially Blocks the Central Anticonvulsant and Bladder Motility Effects of Mu Agonists in the Rat

F. Porreca and F. Tortella

A number of laboratories have shown that kappa agonists block some, but not all, of the pharmacological actions of classical mu agonists (Wood, Drug Dev. Res., 4:429, 1984). We studied the mu antagonist properties *in vivo* of U50,488H, a selective kappa agonist, using elevation of seizure threshold (ST) and suppression of bladder motility (BM) as central mu-opioid endpoints. Anticonvulsant properties of the mu agonists [D-Ala^2, NMePhe^4, Gly-ol]enkephalin (DAGO) and etorphine (ETOR) were studied in male S.D. rats by as latency to seizure onset (ST) induced by flurothyl. Bladder effects of DAGO and ETOR were studied in female S.D. rats as the duration of inhibition of volume-initiated BM. Intracerebroventricular (i.c.v.) DAGO (1.13 ug) or ETOR (1.0 ug) increased ST to 534 ± 34 sec and 484 ± 29 sec, respectively, when compared to control values (357 ± 8 sec); U50,488H (10 ug, i.c.v.) had no protective effect (357 ± 18 sec). Likewise, i.c.v. DAGO (0.0075 ug) and ETOR (0.0018 ug) suppressed BM for 22.8 ± 1.7 min and 21.9 ± 3.1 min, respectively; i.c.v. saline or U50,488H (10 - 60 ug) were ineffective. Antagonist properties of U50,488H on the centrally-initiated effects of DAGO and ETOR were determined for both endpoints in rats pretreated with saline or the kappa agonist. Although lacking agonist activity, prior U50,488H treatment (10 ug, i.c.v.) blocked the ST elevation by i.c.v. ETOR (419 ± 19 sec, $P<0.05$), but not that by i.c.v. DAGO (503 ± 22 sec). Similarly, pretreatment with U50,488H (10 ug, i.c.v.) antagonized the suppression of BM by i.c.v. ETOR (3.6 ± 2.4 min duration, $P<0.01$), but not that by i.c.v. DAGO (23.3 ± 1.6 min duration). Thus, in two endpoints of central opioid activity, U50,488H was found to selectively antagonize the effects of ETOR, but not those of DAGO, both agonists at mu receptors. These findings suggest the existence of subpopulations of mu-opioid receptors (isoreceptors) that are differentially affected by kappa agonists and through which, a diverse group of mu agonists may exert similar effects.

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Tolerance and Abstinence to Mu and Kappa Receptor Stimulation of the Hypothalamo-Pituitary-Adrenal Axis

D. Ignar and C. Kuhn

The hypothalamo-pituitary-adrenal axis is stimulated by administration of mu and kappa opioid receptor agonists to rats as evidenced by a rise in circulating ACTH and corticosterone (CS). Following chronic administration, tolerance and withdrawal develop within the HPA axis. The focus of the present work is to elucidate the role of mu and kappa opioid receptors in the adaptations responsible for these phenomena. This is a novel approach to the study of opiate tolerance and dependence since it is an *in vivo* model in which both processes may be studied. Adult male Sprague-Dawley rats were injected with increasing doses of morphine or the kappa receptor agonist U50,488 twice daily for 5 days. Abstinence and tolerance were assessed 12 and 36 hours, respectively, after the cessation of treatment. The rats were decapitated and blood was collected for measurement of CS by radioimmunoassay. Complete tolerance to a 10 mg/kg challenge dose of morphine was observed after 5 days of morphine treatment (20 mg/kg to 40 mg/kg). The abstinence-induced stimulation of CS which peaks 10 to 14 hours after morphine administration, is observed after 1 day of treatment, before substantial tolerance develops. After the fifth day of treatment, abstinence-induced CS secretion was maximal and could be totally suppressed by 5 mg/kg morphine, a dose to which the HPA axis is completely tolerant. No evidence of cross-tolerance to U50,488 was observed, and this drug did not suppress morphine abstinence-induced increases in CS secretion. Chronic treatment with U50,488 (1 mg/kg to 5 mg/kg) resulted in approximately 70% tolerance to a 1 mg/kg challenge dose of U50,488. Cross-tolerance to 10 mg/kg morphine was not observed. Withdrawal from U50,488 was not associated with elevation of CS levels. These results demonstrate that tolerance to opiate effects on the HPA axis occurs at both mu and kappa receptors. In contrast, abstinence-induced rises in CS secretion seem to be related only to the mu receptor. The mechanism by which abstinence and tolerance to morphine-induced rises of CS secretion develop may be dissociable, as abstinence is observed before measurable tolerance occurs. Tolerance might result from adaptations at hypothalamic sites directly involved in regulation of CRF secretion. Abstinence secretion of CS may be integrated at other neural centers which are known to exhibit hyperactivity during opiate withdrawal.

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Tolerance Develops to Naloxone-Induced LH Secretion in Neonatal Female Rats

E. Field, M. Doron and C. Kuhn

Tonic endogenous opioid peptide (EOP) inhibition of luteinizing hormone (LH) secretion is especially strong in neonatal female rats. Acute or repeated treatment with the opiate antagonist, naloxone (NAL), is followed by an immediate marked rise in serum LH and by a rebound suppression 6 hr later. These findings suggest that a recent report of precocious puberty following chronic NAL treatment of neonatal female rats might be explained by the immediate LH effects of repeated NAL exposure. The current study tests the hypothesis that chronic NAL causes repeated rises in LH associated with accelerated pubertal development. NAL (2.5 mg/kg sc) or saline (SAL) was administered every 6 hr from 1 to 10 days of age. At 10 days of age, pups were challenged with NAL 6 hr after their last chronic injection. Blood samples were obtained by decapitation 20 min later. Some animals were tested for MOR-induced antinociception 6 hr following the last NAL injection. Other chronically treated pups were examined daily for vaginal opening and subsequent vaginal cytology. Whereas in NAL-naive animals NAL challenge produced a 5-fold rise in LH, animals chronically exposed to NAL did not demonstrate an LH rise in response to NAL. Furthermore, LH levels in chronic NAL rats were at basal, not suppressed, levels 6 hr post-NAL. These results suggest that tolerance develops during chronic neonatal NAL treatment to EOP blockade involved in the immediate rise and the rebound suppression of serum LH and might reflect endogenous responses to chronic antagonist treatment, such as EOP receptor up-regulation, changes in EOP neuron activity, or adaptations of the entire hypothalamic-pituitary-ovarian axis. Also, NAL did not block MOR-induced antinociception 6 hr later. Finally, we found no effect of chronic neonatal NAL treatment on the timing of vaginal opening or the first diestrus, in contrast to previously published reports.

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Early Maturation of Mu and Kappa Opiate Control of Hormone Secretion and Effects of Perinatal Opiate Addiction

C. Kuhn, D. Ignar, L. Bero, S. Lurie and E. Field

The significant and specific role of each endogenous opioid peptide on neuroendocrine function suggest that hormone secretion could be altered markedly by perinatal opiate addiction. To investigate this possiblity, we first investigated the ontogeny of mu, kappa and delta-specific endocrine responses in neonatal rats. The effects of chronic treatment then were assessed by evaluating the endocrine response to opiate challenge after chronic administration.

High doses of exogenous (methadone, 2.5 mg/kg) or endogenous (beta endorphin) opiates to 5 day old rat pups elicited endocrine response resembling those observed in adults: increases in prolactin (PRL), growth hormone (GH), corticosterone (CS) and suppression of thyroid stimulating hormone (TSH). Administration of agonists with relative specificity for *mu* (low dose (5 mg/kg) morphine, morphiceptin (0.5 ug i.c.v.) *kappa* (U50,488, 1 mg/kg s.c., dynorphin, 1 ug i.c.v.) or *delta* (D-pen2-pen5 enkephalin, 1 ug i.c.v.) receptors revealed that mu and kappa receptors modulated PRL, TSH and CS secretion as early as postnatal day 5. Furthermore, opiate receptor antagonists naloxone (0.1 mg/kg) and the kappa antagonist MR2266 significantly elevated LH secretion. Kappa receptor stimulation actually decreased GH secretion, while delta and possibly mu receptor stimulation increased GH secretion. These results demonstrate the presence of multiple opiate controls of hormone secretion at an age at which opiate-induced analgesia is still immature.

Although tolerance of opiate-induced CS secretion was not observed after chronic U50,488 administration, chronic morphine (5 to 25 mg/kg, bid. for 5 days) resulted in a marked decrease of CS responses to subsequent morphine challenge, as well as an abstinence-related rise 12 hours after the last dose. This tolerance suggests that endocrine function might both be a target of and a useful experimental system for studying perinatal opiate addiction.

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A Comparison of the Modes of Interaction of (+)- and -(-) Opiates at PCP Receptors

A. Newman, V. Bykov, R. Rothman and K. Rice

Stereospecific binding of [^{3}H]PCP to central PCP binding sites has been established and recent evidence, based on drug discrimination studies, suggests that the (+)-stereoisomers of some opiates share certain effects with PCP, and these effects are not observed to the same degree with the (-)-stereoisomers. The natural (-)-opiates have proven invaluable in elucidating μ opioid receptors. The few (+)-opiates that have been examined have been found to be inert at classical opioid receptors. However, the lack of availability of a variety of unnatural (+)-opiates has precluded extensive study of the pharmacology of these compounds. The recently developed NIH Total Opiate Synthesis has enabled the preparation of many previously unavailable (+)-opiates, for study. Thus synthesis of (+)-morphine, (+)-naloxone, and (+)-naltrexone has been accomplished and these compounds, their natural (-)-stereoisomers, as well as several available opiate enantiomeric pairs were evaluated to determine their mode of interaction at the rat brain PCP receptor, *in vitro*, using ligand binding techniques. Two models were compared: a competitive model, which assumes that the test drug competes directly with the PCP binding site, and a noncompetitive model which assumes that the test drug competes indirectly for the PCP binding site, acting to decrease the B_{max}. [^{3}H]TCP was used to radiolabel PCP binding sites in rat brain. Most (+)-opiates demonstrated higher binding affinity (lower Kd values) to the PCP receptors than their (-)-stereoisomers. The competitive and noncompetitive models of binding fit the data equally well for the enantiomeric pairs of morphine, naloxone and naltrexone, whereas the data for all other compounds tested fit the competitive model over the noncompetitive model of binding. Subsequent behavioral testing will determine whether these compounds are acting as PCP receptor agonists or antagonists.

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(+)-N-Allylnormetazocine Binding Sites: Characterization with *In Vitro* and *In Vivo* Methods

D. Compton and B. Martin

The distinct patterns of pharmacological effects observed with various opioids has led others to postulate the existence of multiple subpopulations of receptors, and to classify the benzomorphan N-allylnormetazocine (NANM, or SKF 10,047) as the prototypic agonist of the σ opioid receptor. Evidence suggests that NANM and phencylidine (PCP) may produce similar effects via a common site of action. Additionally, though comparison of the similarities between (+)- or (-)-NANM and PCP in the production of pharmacological effects depends on the specific test and the species used, in all cases (+)-, but not (-), NANM resembles PCP. The *in vitro* binding of both isomers of ^{3}H-NANM indicates that the isomers bind to distinct sites, and also suggests that it is (+)-, not (-), NANM which is PCP-like. However, the *in vitro* binding of both PCP and NANM is a function of the assay conditions (buffer, ionic strength, ions present, *etc.*), yet the extent to which such factors alter the binding of (+)-^{3}H-NANM *in vivo* are unknown. By using *in vivo* binding methods, the characteristics of saturability, regional distribution, and selective displacement for (+)-^{3}H-NANM binding to mouse brain homogenates has been determined and compared to *in vitro* methods. The *in vivo* (+)-^{3}H-NANM saturation (0.1 µg/kg - 1 mg/kg) and displacement (5 µg/kg - 25 mg/kg unlabeled (+)-NANM) curves are monophasic (R=0.98). Therefore, if (+)-NANM interacts with multiple sites, then the isomer must do so with identical affinities under *in vivo* conditions. *In vivo* (+)-^{3}H-NANM binding (182 µCi/0.85 µg/kg) is inhibited by PCP (75% at 25 mg/kg), (+)-ketocyclazocine (88% at 10 mg/kg), and haloperidol (86% at 10 mg/kg), and is inhibited by naloxone (26% at 1 mg/kg) though not in a dose-responsive fashion, but is not inhibited by (-)-NANM (50 µg/kg) or (-)-ketocyclazocine (10 mg/kg). Additionally, the displacement of (+)-^{3}H-NANM from various brain areas (given below) by these drugs is similar to that observed in whole brain. Lastly, the rank order of the distribution of *in vivo* and *in vitro* (+)-^{3}H-NANM binding is identical, with the exception of the cerebellum which contains the largest number of sites in the *in vivo* assay. A rank order of highest to lowest *in vitro* (+)-^{3}H-NANM binding is: medulla, midbrain, hypothalamus, cerebellum, cortex, striatum, and hippocampus. The (+)-^{3}H-NANM site described in this report is similar to the site(s) previously reported for *in vitro* (+)-^{3}H-NANM binding and refered to as a σ/PCP site. This site may represent a "σ receptor" of unknown pharmacological significance, or the σ/PCP, σ/haloperidol, or "high affinity" σ receptor reported by others.

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Effects of Amfonelic Acid, A Dopamine Agonist, and Morphine on Brain Stimulation Reward

S. Izenwasser and C. Kornetsky

Many drugs of abuse, including stimulants such as cocaine and amphetamine, and opioids like morphine and heroin, will lower the threshold at which rats will work to receive electrical stimulation to the medial forebrain bundle-lateral hypothalamus (MFB-LH). The underlying mechanisms by which this occurs are not completely understood, however there is considerable evidence suggesting that dopamine plays a major role in mediating the reinforcing effects of these drugs. The present study was conducted to investigate the effects of amfonelic acid, a selective dopamine agonist, alone and in combination with morphine, on the threshold for rewarding brain-stimulation.

Bipolar stainless steel electrodes were stereotaxically implanted into the MFB-LH of male F344 rats (Charles River Laboratories). The animals were then trained on a discrete trial procedure to turn a wheel manipulandum in order to receive electrical stimulation to this brain site. Reward thresholds were determined using a modification of the psychophysical method of limits. As in many previous reports, morphine lowered the reward threshold. Amfonelic acid, as in a recent study (Knapp et al., Pharmacologist, 28:150, 1986), also caused threshold lowerings, and these were of a greater magnitude than those seen with morphine. When a low (ineffective) dose of amfonelic acid was administered concomitantly with morphine the threshold lowerings observed were larger than those seen with either drug alone.

These findings suggest that amfonelic acid has abuse potential and that its reinforcing effects may, in fact, be even greater than those of the opioids. Further, these results support the hypothesis that dopamine plays a significant role in mediating brain-stimulation reward and that there is an interaction between opioid and dopaminergic systems.

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Antidepressants in Mouse Narcotic Dependence Tests

W. Schmidt and L. Cook

Amitriptyline, imipramine, and other antidepressants have gained increasing acceptance in the treatment of chronic pain (S. Butler, Adv Pain Res Ther 7: 173, 1984). Previous studies have shown that selected antidepressants produce naloxone-reversible analgesia or morphine cross-tolerance in the mice, suggesting an opioid-type analgesic component to their action (M.C. De Felipe et al., Eur J Pharmacol 125: 193, 1986). Antidepressants used in these tests also produce motor incoordination which makes interpretation of their effects less certain. Accordingly, we examined the activity of sedating and non-sedating antidepressants in several analgesic tolerance and dependence procedures commonly used to investigate the action of narcotic drugs. Using inhibition of phenylquinone writhing to measure analgesic activity, analgesic tolerance studies show that nomifensine, a non-sedating antidepressant, is fully cross-tolerant to morphine in morphine-tolerant mice. Amitriptyline and trazodone showed a partial cross-tolerance to morphine that might have been greater had the drugs not caused strong sedation and motor impairment at higher test doses. We observed a reduction in these overt behavioral signs in morphine-tolerant mice, indicating side-effect cross-tolerance in addition to analgesic cross-tolerance. In the Single Dose Suppression test (partially-withdrawn morphine-dependent mice), nomifensine and trazodone suppressed morphine withdrawal jumping but not autonomic signs of withdrawal. Amitriptyline exacerbated withdrawal jumping. Morphine suppressed all withdrawal signs. In a 72 hr dependence induction test, mice treated with continuous infusions of amitriptyline or trazodone showed a high incidence of withdrawal jumping responses upon discontinuation of the minipump infusion. However they had none of the characteristic autonomic withdrawal signs shown in morphine-treated mice. Mice treated with chronic infusions of chlorpromazine or haloperidol also showed abrupt abstinence withdrawal jumping. Naloxone was ineffective in precipitating withdrawal in non-withdrawn mice infused with amitriptyline, trazodone, chlorpromazine, or haloperidol. These results demonstrate that anti-depressants and other CNS-active drugs produce some tolerance or dependence-related signs similar to those of narcotic-related analgesics, but do not show all signs characteristic of narcotic-like activity in mice. Therefore, some commonly observed withdrawal signs are not restricted to the use of typical opioid analgesics.

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Calorimetric Analysis of ICV Morphine in the Rat

A. Zwil, T. Lynch, R. Martinez, E. Geller and M. Adler

Body temperature (Tb), 02 consumption (V02) and heat loss (Q) were continuously recorded in a whole-body calorimeter maintained at 20°C before and after intracerebroventricular (ICV) injection of 30 µg morphine sulfate (MS) in awake, unrestrained, young adult, male Sprague-Dawley rats. MS was injected via a cannula chronically implanted in the right lateral cerebral ventricle.

ICV MS produced a sustained *hyper*thermia in all 7 animals tested. Tb began to rise between 0.25 and 3.25 (µ±SE = 2.0±0.42) hours post-injection, peaked at 1.5 to 3°C above baseline at 2.25 to 4.25 (µ±SE = 3.14±0.29) hours, and remained at or near that level until the end of the recording session. In the longest session recorded, Tb was still elevated at 3 hours after onset of hyperthermia. In five out the seven cases hyperthermia was accompanied by an increase in V02, while an increase in Q did not begin until 30 to 60 minutes after onset of hyperthermia. The net result of the changes in V02 and Q was that the Q/V02 ratio, an index of thermoregulatory activity, usually decreased simultaneously with the hyperthermia (due to the increased V02) and returned to near baseline after the subsequent increase in Q. These results show that the hyperthermia was caused by an increase in metabolic heat production, and that thermoregulatory responses were subsequently activated in an attempt to stabilize Tb at a higher level. Four animals injected with saline instead of MS showed no change in Tb, and only transient fluctuations in Q and V02.

In previous work from this laboratory, low doses of MS injected subcutaneously (SC) produced *hyper*thermia with a decrease in Q/V02, while high doses of MS produced *hypo*thermia accompanied by an initial increase and followed by a subsequent decrease in Q/V02.[1] In the current investigation, MS administered ICV produced effects upon Q and V02 similar to a hyperthermic SC dose, indicating that the mechanism of action is the same for both routes. These results are in accord with the hypothesis, advanced previously, that MS causes hyperthermia by acting upon *mu* receptors in the brain, and hypothermia by activating *kappa* receptors in the spinal cord or periphery.[2] (Supported in part by grant #DA00376 from NIDA.)

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Tolerance to Fentanyl and Cross Tolerance to Morphine in a Drug Discriminative Procedure

M. Emmett-Oglesby and A. Herz

This investigation tested the hypothesis that tolerance would develop to the discriminative stimulus properties of fentanyl upon discontinuation of discrimination training and injection of fentanyl in doses larger than the training dose. Rats were trained to discriminate fentanyl, 0.04 mg/kg, from saline using a two-lever choice procedure with food as a reinforcer. Subsequently training was stopped, and fentanyl, 0.08 mg/kg, was injected every 12 h for one week. This procedure did not produce tolerance, nor did tolerance occur when fentanyl, 0.16 mg/kg every 24 h, was continued for an additional week. In contrast, a dose of morphine (8.0 mg/kg) equated for efficacy to the 0.08 mg/kg dose of fentanyl, produced both tolerance to the morphine stimulus and cross-tolerance to the fentanyl stimulus after three to four days of administration. In an additional experiment, the time course for detection of fentanyl was found to be significantly shorter than the time course for the detection of morphine. Thus, these results, as well as a previous report (Colpaert et al. Neuropharmacology. 17: 705-713, 1978) of failure to find tolerance to fentanyl, are perhaps attributable to fentanyl's brief duration of action. This hypothesis predicts that tolerance should be observed if the frequency of administration of fentanyl were increased. In a subsequent study, when 0.12 mg/kg of fentanyl was administered every 6 h for 4 days, tolerance to fentanyl and cross-tolerance to morphine was found. In addition, sensitivity to fentanyl spontaneously reverted to baseline within 4 days of terminating chronic fentanyl administration. These data indicate that tolerance occurs to fentanyl in the drug discrimination procedure, and they suggest that pharmacokinetic factors may account for some failures to find tolerance.

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Regression Analysis as a Measure of Tolerance Development to Opioid-Induced Disruption of Food-Maintained Responding in Macaque Monkeys

S. Lukas, N. Mello, M. Bree and J. Mendelson

This study assessed the development of tolerance to opioid-induced disruption of food-maintained responding using regression analysis techniques. Single daily subcutaneous injections of buprenorphine (1.0 mg/kg), diprenorphine (1.0 mg/kg), or heroin (1.0 mg/kg) were given over 25 consecutive days to examine the degree and the rate of tolerance development to drug-induced suppression of food-maintained responding. Similar techniques were applied during saline substitution for an additional 15 days. One gram food pellets were available on a second order schedule (FR 4 VR 16:S) during 1-hr sessions three times a day (7:00 a.m., 11:00 a.m., and 3:00 p.m.). Daily drug and saline control injections were given at 10:00 a.m. During the first three days of treatment all three drugs produced marked suppression of food-maintained performance. Recovery from buprenorphine- and diprenorphine-induced suppression of food-maintained responding occurred within four and eight days, respectively. By the 25th day of buprenorphine and diprenorphine treatment, operant responding for food increased significantly above control levels ($P < .01$). In contrast, heroin-induced disruption of food-maintained responding recovered only slightly and remained significantly reduced ($P < .01$) throughout the 25-day treatment period. Regression analysis of the linear portion of the time-effect curve revealed significant differences in both the rate and the degree of tolerance development to these three opioids. The buprenorphine and diprenorphine recovery curves were parallel while the slope of the heroin recovery curve was not significantly different from zero. Saline substitution for all three drugs resulted in a gradual return to control levels of food pellets earned (i.e., increased responding after heroin and decreased responding after buprenorphine and diprenorphine). These differences in tolerance development may reflect pharmacokinetic differences between the relatively short-acting heroin and the longer-acting diprenorphine and buprenorphine.

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Comparison Study of Oral Pentazocine, Pentazocine + Naloxone and Placebo in Postepisiotomy and Postoperative Pain

A. Sunshine, N. Olson, R. Axtmayer, I. Ramos and E. Laska

Our objective was to study the effect of the addition of 0.5mg naloxone (NX) to Pentazocine 50mg on the analgesic efficacy of pentazocine 50mg. A single oral dose of either pentazocine, pentazocine-NX or placebo was given to 48 patients with moderate pain and 76 patients with severe pain resulting from episiotomy, cesarean section or gynecological surgery in a randomized double-blind parallel groups study. Patients were evualted over a 4-hour period. An analysis of the entire population showed both pentazocine and pentazocine-NX to be significantly more effective than placebo for all measures. In addition, pentazocine-NX was significantly less efficacious than pentazocine for %SPID and for relief and PID at the fourth hour. Because there was a significant interaction between drug and baseline pain intensity for the variable SPID, comparisons were made between pentazocine and pentazocine-NX within each baseline pain intensity group. For patients with moderate baseline pain, pentazocine-NX produced significantly less pain relief than pentazocine for SPID and for relief and PID at hours 3 and 4. In patients with severe baseline pain, there was no significant difference between pentazocine and pentazocine-NX.

Four patients complained of adverse effects; 3 received pentazocine and 1 patient received pentazocine-NX as the treatment.

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Buprenorphine: Pilot Trials in Borderline Patients and Opiate Dependence - Treatment of a Common Disorder?

R. Resnick and F. Falk

Buprenorphine (BUP), a morphine-type mixed agonist/antagonist, has low dependence liability and toxicity, with a pharmacological profile determined primarily by partial agonism and slow kinetics at u-receptors. These features, plus clinical reports indicating acceptance by heroin addicts and antidepressant activity, suggest it be evaluated for use in treating psychiatric disorders. Two groups were studied.

In Group A, 15 symptomatic patients in ongoing psychotherapy, received a sublingual test dose BUP 0.2 mg. Positive responders (N=9) all met DSM-III criteria for borderline personality disorder (BPD), while negative responders (N=6) all had other diagnoses. Symptom severity scores, BUP vs. placebo (PL), were reduced by a mean of 43% and 50% respectively, in 5 ss who received, single-blind, BUP (0.3-1.2mg IM) or PL daily for 9-14 days in an ABA design (A=PL; B=BUP) and 4 ss who received, in 2 sessions, BUP and PL administered 1-2 hours apart, in counterbalanced order. Hamilton depression scores were reduced by 30-50% (mean 13.9 to 5.7), after 1 month on daily self-administered BUP. Subjects reported a profound sense of feeling better, but upon discontinuing BUP, symptoms returned immediately and most subjects asked to continue treatment.

In Group B, 20 opiate dependent subjects, in various stages of withdrawal, received IM BUP (0.3-1.2mg) and, after 1-2 hours, mean abstinent severity scores were reduced by 65%. Eight ss completed detoxification (14 days BUP + 7 days PL). Except for 4 ss who started naltrexone, all others met DSM-III criteria for BPD. Twelve subjects received maintenance BUP (0.6-3.0mg/day); 5 were terminated for illicit drug use, 7 were drug-free at 6-9 months.

Diagnostic comparisons between negative and positive responders to BUP and the favorable clinical results, support the idea of using BUP to identify and treat a homogeneous subgroup within the borderline personality disorder category. Guidelines need to be developed for using BPN in clinical psychiatry.

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Serologic Investigations in Parenteral Drug Abusers

L. Brown, B. Primm and D. Murphy

The prevalence of the acquired immunodeficiency syndrome (AIDS) and related infections has been associated with exposure to the human immunodeficiency virus (HIV). Previously, we have shown that intravenous drug abusers (IVDAs), the second largest risk group for the development of AIDS, exhibit derangements in immunologic function that are associated with various patterns of drug abuse. However, the relationship of underlying immunologic function to subsequent HIV infection remains unclear. We examined the seroprevalence of infection by pathogens often associated with HIV infection and other immunologic markers in 384 newly enrolled patients in methadone maintenance clinics in New York City and 59 healthy volunteers.

Chi-Square analyses were conducted for the presence of antinuclear antibody (ANA) and rheumatoid factors and for abnormal levels of titers of antibody to *Toxoplasma gondii* (TG), cytomegalovirus (IgG and IgM), and herpes simplex virus (type 1 & 2) in enrollees as compared to controls and in relationship to HIV seropositivity.

The results demonstrated that the prevalence of abnormal antibody titers was greater in the enrollees, reaching statistical significance in ANA ($p<0.001$), and TG ($p<0.001$). These abnormal serological results were not associated with HIV seropositivity in the enrollees. These findings suggest that intravenous drug users incur a greater prevalence of alterations in these serological markers, suggestive of immunologic dysfunction yet unrelated to previous HIV exposure.

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Clonidine and Naltrexone: Rapid Treatment of Opioid Withdrawal in the Outpatient Setting

E. Vining, T. Kosten and H. Kleber

Clonidine hydrochloride (an alpha-2 adrenergic agonist) and naltrexone hydrochloride (an opioid antagonist), given in combination, provide a safe and effective treatment of abrupt opioid withdrawal over four or five days in an outpatient setting. Following a naloxone challenge test to verify and quantify opioid dependence, 14 of 17 (82%) heroin users successfully withdrew from opioids and attained maintenance levels of naltrexone. Eight of nine (89%) successfully completed the five day study in which naltrexone therapy was begun on day two. Six of eight (75%) successfully completed the four day study in which naltrexone therapy was begun on day one. Three to five days of clonidine hydrochloride treatment with a peak mean dose of 0.6 mg/day on day two for the patients in the five day study, and 0.5 mg on days one and two for patients in the four day study, attenuated the withdrawal-inducing effects of naltrexone. Both groups received naltrexone in single morning doses which were rapidly increased from 12.5 mg on the first day of naltrexone therapy to 50 mg on the third day. Significant correlations were observed between naloxone challenge test score and observer-rated symptomatology during treatment. Clonidine significantly decreased blood pressure in both groups without producing clinical problems. This study has improved the availability of the clonidine- naltrexone combination by developing a single dose per day naltrexone regimen with naltrexone doses generally available to any opioid treatment facility.

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Doxepin Treatment of Depressed Opiate Addicts Undergoing Methadone Detoxification

S. Batki, S. Wheeler, R. Jones, J. Sorensen and K. Brennan

Eighty-one opiate addicts with DSM-III Major Depressive Disorder underwent 21-day outpatient methadone detoxification and were concurrently treated with a seven-week course of doxepin or placebo in a double-blind randomized clinical trial.

Preliminary analysis of the full sample showed differences in treatment outcome between the doxepin and placebo groups. Addicts treated with doxepin improved more than those receiving placebo. Improvement was consistently greater in the doxepin group in depression, opiate use, opiate withdrawal symptoms, and craving for opiates. These outcome differences approached statistical significance for most of the measures. Improvement in the doxepin group began to reach statistically significant differences over placebo at Week 3, when methadone detoxification was completed. This pattern of improvement is consistent with the expected onset of antidepressant treatment effectiveness. Attrition posed problems for both the doxepin and control groups, yet about half of subjects succeeded in completing the seven weeks of treatment and reached the final 12-week follow-up. While serum doxepin levels were low in the subsample of subjects in whom this measure was obtained, medication compliance was judged to be satisfactory as measured by the consistently higher levels of antidepressant side effects reported by the doxepin group.

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From Methadone Maintenance to Abstinence: Evaluation of an Outpatient Tapering Network

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and L. Wermuth

This is the first outcome report of a study that investigated the impact of providing tapering and aftercare assistance to heroin addicts enrolled in methadone maintenance. In an earlier effort our group had demonstrated the efficacy and limitations of providing such assistance through concurrent treatment in a therapeutic community (Sorensen, et al., 1984); the current effort extended this approach to outpatients. A pre-post, matched comparison group design assessed the program's impact on patients' ability to achieve detoxification from methadone, their drug and alcohol usage, treatment progress, treatment satisfaction, severity of problems associated with addiction, and assimilation into the non-drug-using community. Fifty-eight patients in three Experimental group clinics received an enriched tapeing and after-care program, while 56 patients in three matched Comparison clinics received written materials only. Of the 114 subjects, 101 were reached for a follow-up interview three months after study termination (89%).

Repeated measures analyses of variance generally did not reveal differences between the Experimental and Comparison conditions. The Experimental group had been more dysfunctional at study entry, but results showed few differences between the two groups on the outcome measures. Twenty-nine percent of the Experimental subjects and 20% of the Comparison subjects tapered off methadone during the study. The study results underscore the difficulties that heroin addicts have in tapering off methadone to a drug-free lifestyle.

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LAAM Maintenance for Opioid Addicts Who Cannot Maintain with Methadone

F. Tennant, Jr.

Ten heroin addicts who could not adequately maintain on a high daily dosage of methadone were studied. They demonstrated continuous drug and/or alcohol abuse and showed low or non-detectable plasma concentrations (mean 38.9 ± 63.3 S.D. ng/ml) 24 hours after a 100 mg oral dose. Urine testing of these individuals revealed that 66.4% of the samples showed use of cocaine, heroin, or benzodiazepines. Nine of the ten (90%) were able to maintain with LAAM as evidenced by lack of drug/alcohol abuse and adequate plasma concentrations of LAAM metabolites. The mean plasma concentrations of NOR-LAAM and DI-NOR-LAAM were 201.6 ± 255.0 S.D. ng/ml and 160.2 ± 62.3 S.D. ng/ml respectively. Opioid addicts who cannot successfully maintain with high dosages of methadone should attempt LAAM maintenance due to LAAM's different metabolism.

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Differences Among Treatment Clinic Types in Attitudes Toward Narcotic Addiction

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and P. Stephenson

The present study examined treatment related attitudes and expectations across drug abuse clinics classified according to the number and types of treatment modalities offered. Assessment of the attitudes and expectations of respondents concerning narcotic addiction and its treatment was accomplished by the use of a 142-item questionnaire whose content areas included four major domains: the nature of addiction; the origins of addiction; ways of dealing with addiction, including treatment; and the personalities and characteristics of addicts in general. Data were collected on 783 clients and 230 agency personnel in 24 drug treatment clinics in five states. A components-type factor analysis of the entire set of items for an original sample of 1137 respondents (clients and staff) was performed to determine the major content dimensions of the questionnaire. Of the ten factors identified, three were measures of attitudes and perceptions more directly relating to the treatment process: ExAddict Counselor Good/Methadone Bad; Addicts Can Change; and Group Treatment Good. Two additional factors, Drugs OK and Addicts Need Control, also appeared likely to have treatment implications that would be reflected in drug abuse intervention strategies. For these dimensions, differences were determined among clients and staff according to type of treatment clinic (three types involving the provision of methadone maintenance in various combinations with other treatments and one involving the use of abstinence only). The most pronounced differences were between the methadone clinics and those offering abstinence only. Both the clients and staff of abstinence clinics were more skeptical concerning treatment effectiveness, were more negative regarding the use of narcotic drugs, and were more disposed to the use of ex-addict counselors and group procedures in treatment.

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Treatment of the Dually Diagnosed Substance Abuser

H. Weiner and M. Wallen

Definition: Patient who is addicted and also has a clinically significant psychiatric disorder.

Major Considerations:

1. Psychopathology is a definite risk factor for addiction.
2. Psychopathology may precede addiction or may develop as a result of addiction.
3. Some portion of substance abuse in almost all addicts is an attempt to self-medicate discomfort associated with life stress or psychological problems.
4. The degree of psychiatric impairment will affect treatment outcome and post-treatment success. In general, the higher the level of impairment, the less favorable is the treatment outcome.
5. Untreated psychopathology greatly increases the probability of relapse.
6. Accurate diagnosis and effective treatment of psychopathology might prevent the behavioral problems which commonly result in patients being discharged for "treatment resistance".

Eagleville's Treatment Model

1. Comprehensive Assessment - evaluations by a physician, nurse, psychologist, social worker, adjunctive therapist and psychiatrist.
2. Individual Treatment Plan, utilizing results of assessments and patient's own goals. Components of treatment are individually prescribed based on the patient's abilities and limitations.
3. Psychiatrist, as clinical leader of the team, provides guidance and direction in treating the addiction and the psychiatric impairment.
4. Components of treatment include group therapy (major modality), individual and family therapy, art and horticulture therapy, assertiveness and relaxation

training, vocational and educational guidance, social skills development, leisure time education, arts and crafts, physical activities, AA/NA/CA meetings, and educational seminars.

5. Aftercare planning - as with any chronic disease, this is the critical component. Continued treatment for both substance abuse and psychiatric problems must be implemented, as well as AA, NA, or CA.

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Contingency Management with a Poly-Drug Abuse Methadone Maintenance Population: Take-Home and Dose Incentives

M. Iguchi, M. Stitzer, G. Bigelow and I. Liebson

Previous studies have shown that take-home (TH) incentive programs based on urinalysis (UA) results can reduce illicit drug use among methadone maintenance (MM) clients. This study sought to determine whether additional therapeutic benefit could be obtained by adding a negative incentive dose reduction procedure to a TH incentive program. Sixteen polydrug abusing MM clients were randomly assigned to 1 of 2 treatment (TX) conditions. In both TXs, TH doses were available for drug-free urines while in one TX methadone dose was also reduced as a consequence of multiple drug-positive UA results. The number of TH's available each week to study subjects was increased by 1 for each 2 consecutive weeks (x3 weekly testing; broad-spectrum TLC) of drug-free results, up to 3 TH's per week. The number of TH's was reduced by 1 for each urine sample that tested positive for any illicit substances. The dose change and TH contingencies operated independently. All dose alterations were in increments of 10% of the original dose. Two or three drug-positive samples resulted in a dose reduction, 1 drug-positive sample had no effect on dose and 0 drug-positive samples resulted in a dose increase. Dose decreases continued until a 0 mg dose was reached (clients could remain in TX at 0 mg methadone) while dose increases could not exceed the original dose. A significant TX effect was noted ($F=11.11$; $p<.005$). Only 8% of the UA results were drug-free during a 12-week baseline period for all subjects while 42% of the UA results were drug-free during the 20 weeks of TX intervention (missing data from study dropouts replaced by average % drug-free during the 6 weeks prior to dropout). No group or group by time effect was noted. While there were an equal number of successful and failed cases in the two study conditions, a significant difference was noted in the final disposition of the TX failures from each group. Three of five TX failures in the TH only condition remained at the clinic using drugs, while all 5 TX failures in the dose incentive condition were no longer in TX ($z=3.53$; $p < .001$). TX outcome results are consistent with previous work from this laboratory showing that positive reinforcement interventions as compared to aversive control procedures can produce an equivalent number of TX successes while avoiding dropout among clients who fail to respond to TX.

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Identification of Personality Disorder Subtypes Among Drug Abusers Using the Million Clinical Multiaxial Inventory

D. Calsyn and A. Saxon

The high prevalence rates of DSM-III Axis II disorders in drug abusing individuals have previously been established. The Millon Clinical Multiaxial Inventory (MCMI), a relatively new personality assessment tool, purports to assess Axis II as well as Axis I disorders. This research investigated the frequency of MCMI Axis II subtypes among drug abusers. The MCMI was administered to 45 opioid addicts and 31 polydrug abusers at the VA Drug Dependency Treatment Program, Seattle. The mean age of patients was 36.8 (SD=7.6); the racial make up, 78% White, 19% Black and 3% Hispanic. The MCMI profiles were sorted four times identifying profiles indicating: 1) the most severe Axis I psychopathology, 2) some affective/somatic disturbance, 3) more serious personality disturbance and, 4) as one of 5 categories based on 3 high point codes for the 8 basic personality scales. Categories based on authors' clinical experience and cluster analysis reported previously were: (a) narcissistic/antisocial, (b) negativism/anxiety/social withdrawal, (c) dependent, (d) no disorder, (e) unclassified. Of the sample 12% exhibited severe psychopathology often associated with psychotic processes. These elevations were more common for polydrug abusers (19%) than opioid addicts (7%, χ^2=2.8, p <.10). Fifty-five percent exhibited affective disturbance (polydrug abusers 71% vs. opioid addicts 44%, χ^2=5.2, p <.05). Severe personality disorder was exhibited in 28% of the cases with no significant difference between polydrug abusers and opioid addicts. Eighty-six percent of all profiles can be placed into one of the first four subtype groups based on the 8 basic personality scales. A contingency table analysis (χ^2=8.1, p <.05) indicates the polydrug abusers (45%) were most likely to obtain the highest elevations on the schizoid, avoidant, and passive-aggressive scales. These individuals are seen as being socially isolated, pessimistic and negative. Others view them as withdrawn complainers. Opioid addicts (38%) were more likely to obtain the highest elevations on the narcissistic and antisocial scales. These people are seen as self centered, with little empathy for others. They often have strong feelings of entitlement and aggressive feelings toward others. These first two subtypes accounted for 61% of all profiles. Another 17% were classified as dependent profiles (opioid addicts 22% vs. polydrug abusers 10%). Eight percent of profiles had no elevations on the basic personality scales.

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Intrathecal Injections: Ca++ Causes Analgesia, Morphine Produces Hypoglycemia

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The acute percutaneous intrathecal (i.t.) administration of calcium (0.24 umole) to ICR mice produced an increased latency in the tail-flick test that was blocked by naloxone (1-2 mg/kg, s.c.). A dose-dependent potentiation of the analgesic effects of i.p morphine or i.t. leu- or met-enkephalin was observed after i.t. calcium. EGTA and verapamil, given i.t., antagonized the analgesic effect of i.p. morphine. These results contrast with previous work showing antagonism of morphine by i.c.v. calcium. Calcium, injected i.c.v., prevented the analgesic effect of i.t. calcium, when equal doses were given by both routes.

The i.t. administration of morphine (12.5-50 ug) caused a dose-dependent hypoglycemia, whereas the s.c. injection of morphine (2.5-80 mg/kg) produced a dose-dependent hyperglycemia. Both effects on blood glucose were antagonized by s.c. naloxone. Naloxone also blocked the lethality, but did not prevent scratching behavior and tonic seizures, caused by high-dose i.t. morphine. Only the lethal effect of high-dose i.t. morphine was partially blocked by loading mice with glucose i.p. Although delayed, the hypoglycemic effect of i.t. morphine was still seen after glucose loading, after induction of diabetes with streptozocin, and after administration to genetically diabetic C57BL/KsJ (db/db) mice. All of these effects of i.t. morphine were blocked by acute spinal transection at T10-T11, but were not mimicked by morphine injected i.c.v. (6.25-50 ug), which showed a bell-shaped dose-response curve for hyperglycemia, without producing any hypoglycemia.

These results indicate a supraspinal/infraspinal dichotomy regarding modulation of analgesia by calcium and modulation of glucose by morphine. It is concluded that 1) the modulation of morphine-induced analgesia by centrally-administered calcium depends upon the route of calcium administration, and 2) the modulation of blood glucose by morphine also depends upon the route of morphine administration.

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Number of Risk Factors Predicts Three-Year Probabilities of Heavy Drug and Alcohol Use in Adolescents

B. Bry, M. Pedraza and R. Pandina

Three year probabilities of heavy drug and alcohol use in adolescents can be predicted by applying a nonlinear, multiple risk factors methodology to current knowledge about precursors. In 1979, 446 adolescents recruited through a random household survey were classified according to the number of 10 established psychosocial risk factors they each displayed. A second assessment in 1982 revealed that 100% of the youth who had displayed 5 or 6 risk factors and 75% of those who had displayed 4 risk ractors in 1979 reported heavy drug or alcohol use by 1982, yielding 90% predictive accuracy. Conversely, 93% of the youth with no risk factors in 1979 reported no heavy use by 1982. The findings support a multiple pathways model of substance abuse in that many disparate combinations of risk factors were associated with heightened risk of heavy use.

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Ethanol Self-Administration in Alcoholic Methadone Patients: Analysis of Drinking Patterns and Evaluation of Behavioral-Pharmacological Treatment

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Alcoholism is a problem frequently encountered in the methadone treatment of narcotic addicts. The methadone clinic is a unique setting for the study of alcohol consumption by virtue of its requirement of frequent patient attendance which permits the intensive assessment of alcohol consumption as indicated by breath-analysis. In this study we report on the assessment of the drinking patterns of alcoholic methadone patients and on a combined behavioral- pharmacological treatme of excessive alcohol consumption. We have intensively assesse the drinking patterns of over twenty alcoholic methadone patients for periods of up to a hundred consecutive clinic visits in some cases. An analysis of those patterns of alcoho consumption shows that the temporal distribution of blood alcohol levels obtained from alcoholic methadone patients is very similar to that reported in studies of alcohol consumptio of alcoholics living on research wards; that is, several days with large quantities of alcohol consumption alternating with several days with small quantities consumed. These results indicate that the findings from laboratory inpatient studies a representative of naturalistic alcohol consumption. Moreover, these results provide evidence that the methadone clinic can serve as a naturalistic laboratory for the study of ethanol self-administration and for the treatment of excessive alcohol consumption. After the assessment of alcohol consumption, we provided treatment of alcoholism consisting of reinforcing disulfiram consumption with methadone clinic privileges. Disulfiram is an effective treatment, but often patients elect to discontinue disulfiram maintenance. By reinforcing disulfiram consumption the problem of patient compliance can b resolved. The results of our study indicate that relative to baseline data the reinforcement of disulfiram consumption results in significant decreases in the number of days drinkin the amount consumed and improved laboratory measures of liver function. Thus, combined-behavioral pharmacological treatment is effective for alcoholic methadone patients.

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Methodological Problems in Psychopharmacological Studies with Alcoholism and Drug Addiction

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Results from early psychopharmacological studies with alcoholism and other addictions are difficult to interpret because they often lacked control groups and standardized instruments for establishing diagnosis and efficacy. This paper addresses issues relating to research design, patient selection, and treatment adequacy and safety in psychopharmacological studies involving alcoholism and the addictions. Use of placebo controls, management of side effects, preservation of the double blind, control of potential confounding variables, compliance with medications, treatment duration, ethical issues, and concomitant therapies are among the topics discussed.

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Opioids' Modulation of Alcohol Intake

L. Reid and C. Hubbell

Results of various procedures designed to assess the effects of administration of opioids on voluntary intake of alcoholic beverages among rats leads to a number of conclusions. Across a wide variety of experimental arrangements including sex, age, housing conditions and nutrient deprivation of rats and including different alcoholic beverages and schedules of presenting beverages (night or day, flavored or unflavored beverage), it is consistently found that injections of small doses of morphine increase rats' intakes of ethanol. Other agonists at opioceptors, such as methadone and fentanyl at small doses, also increment intake of alcoholic beverages. Naloxone, as well as other antagonists at opioceptors, decrease intakes of ethanol. Doses of morphine as low as 0.4 mg/kg, subcutaneously given, reliably increase intakes of sweetened ethanol solutions. Doses of naloxone at 2.5 mg/kg produce as much decrease as larger doses, except very high doses. So, it is clear that opioids modulate intake of alcoholic beverages.

Opioids also modulate intake of other ingesta such as sweet and salty water. Although opioids' modulation is not specific to ethanol solutions, the effects on alcohol-intake can be quite dramatic. Rats fixed with osmotic pumps for delivery of chronic infusion of morphine were given daily opportunities to take, after water-deprivation, either water or a sweetened alcoholic beverage for 1.5 hr/day. These rats, after 3 days of the daily regimen, were taking more ethanol solution than controls. When these same rats were given an injection of morphine after 8 days on the regimen, they took on the average, over 5.0 g of pure ethanol/kg of body weight during 1.5 hr and did so across 8 consecutive days. Other data confirm that morphine's appetitional effects are unrelated to morphine's capability to produce analgesic or narcotic effects. Also, the small-dose-morphine-effect of incrementing intake of alcoholic beverages does not show tolerance. Our results lead to the suggestion that surfeits of opiodergic functioning (as achieved by morphine administration) may be a salient variable with respect to instances of excessive intake of alcoholic beverages.

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Ethanol Withdrawal Anxiety: Characterization in an Animal Model

H. Lal, D. Benjamin, C. Harris, S. Bhadra and
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Ethanol given acutely exhibits a mild anxiolytic action, but chronic ingestation of ethanol produces dependence that is characterized by withdrawal consisting of anxiety-like subjective symptoms. Typically, the subjective effects of withdrawal occur earlier and last longer than overt signs of withdrawal. While the anxiolytic effects of ethanol may contribute to occasional drinking, ethanol-withdrawal anxiety may be critical in the genesis and maintenance of ethanol abuse. Because of their subjective nature, anxiety-like effects have been difficult to study in animal experiments. Our experiments used an interoceptive discriminative stimulus (IDS) produced by an anxiogenic drug, pentylenetetrazol (PTZ) as an index of subjective effects in animals. We proposed that a comparison of the IDS produced by test treatments with the IDS produced by PTZ would serve as a suitable animal model to bioassay subjective effects related to ethanol use or ethanol withdrawal. We measured the efficacy of ethanol in blocking two different levels of PTZ stimulus. Also, we investigated the degree to which withdrawal from ethanol produces an IDS which mimicks that of PTZ in rats previously trained to discriminate PTZ. To date we have observed that ethanol fully blocks only the weaker of two stimuli produced by PTZ. Also, withdrawal in rats given chronically intoxicating doses of ethanol produced a PTZ-like IDS. This stimulus was speciffically associated with ethanol withdrawal and was not a result of stress or handling associated with ethanol administration or its intoxication. The PTZ-like withdrawal stimulus began when ethanol blood levels declined, and disappeared within 2-3 days. The intensity of this stimulus was related to the dose and duration of ethanol administration as well as the duration of abstinence. The withdrawal stimulus was blocked by ethanol or diazepam. Thus, these experiments provide an animal model for objective evaluation of a subjective symptom related to ethanol abuse. Characterization of ethanol withdrawal with respect to its subjective effects offers a new tool to investigate behavioral and neurobiological factors in ethanol dependence and abuse. (Supported by Grant AA06890)

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Effects of Ethanol Self-Administration and Withdrawal in Rats Responding for Food and Water

P. Beardsley, H. Kalant, A. Stiglick and I. Woodworth

Four, adult male Long-Evans rats were trained to lever-press for water, ethanol (8, 16 or 32% w/v) and food-pellet deliveries in a three-lever operant conditioning chamber. Periods of ethanol availability both preceded and followed periods of response-contingent food availability. In Experiment 1, the rats were exposed to seven, 3.5-hr blocks each day, each block sequentially consisted of 30 min of ethanol alone, 1 hr of food, 30 min of ethanol and water, and 1.5 hr of water alone. In Experiment 2, the same schedule was employed but a 7-day ethanol withdrawal period (only water and food were available) followed tests at each concentration. In Experiment 3, both water and an ethanol solution were available in each drinking period; during each day there were 16, 0.5-hr periods of fluid availability separated by 15, 1-hr periods of food availability.

Often more daily ethanol than water deliveries occurred. The number of ethanol deliveries decreased with increases in concentration but the amount of ethanol consumed (g/kg body wt.) increased and reached values as high as 11.5 g/kg/day. Blood ethanol concentrations determined during the final hour of availability of each ethanol concentration across the three experiments ranged from trace amounts to 114 mg/dl, and were observed as high as 135 mg/dl shortly after (i.e., 30 min after) the end of drinking bouts during Experiment 3. The ingestion of small amounts of ethanol tended to increase response rates for food whereas ingestion of progressively larger amounts of ethanol significantly and progressively decreased food response rates. Ethanol self-administration was greater when it followed food sessions than when it preceded food sessions. When ethanol availability was withdrawn, food response rates increased. Tolerance tests administered at the end of Experiment 3 demonstrated that the rats' ethanol self-administration histories had produced tolerance to the hypothermic and the motor incoordination effects of an ethanol test dose (2.5 g/kg i.p.) and that this tolerance was lost after two weeks of abstinence.

The conditions of these experiments satisfy many of the requirements of an animal model of alcoholism and could provide baselines for which to assess specific effects of pharmacological agents on rates of self-administered ethanol.

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Free Choice Consumption of Ethanol by AA and ANA Rats in an Operant Model

H. Kalant

Rats genetically selected for high and low voluntary consumption of ethanol (Alko AA and ANA respectively, kindly provided by Dr. K. Kiianmaa) were trained to lever-press for fluid in the paradigm described by Beardsley et al. (this conference). In a 3-lever operant chamber they learned to press one lever for access to ethanol solution, a second for water, and a third for food. A daily 23-hr schedule consisted of sixteen 30-min sessions in which both fluids were available on a CRF schedule, separated by fifteen 1-hr sessions of pressing a third lever for food pellets [chained FI40' (FR5:S) FR1(20) schedule, maximum of 20 pellets per session or 300 per day]. Each rat was carried through 3 complete cycles, a cycle consisting of at least 6 days of stable responding at each of 3 ethanol concentrations: 8%, 16% and 32% w/v.

AA rats preferred ethanol over water at all 3 concentrations in all 3 cycles, except for the 32% in the first cycle. ANA rats preferred water throughout. However, the absolute amount of ethanol consumed rose with increasing ethanol concentration in both groups, and both showed increasing preference and consumption with 8% ethanol over the 3 successive cycles. The difference between strains remained constant: e.g., at 32% ethanol, AA rats took 10.4 ± 1.4 g/kg/day vs. 5.4 ± 1.0 ANA rats. Tail blood ethanol levels, 30 min after a burst of drinking at 32% ethanol in cycle 3, were 145-270 mg/dL in AA and 0-112 in ANA rats. On completion of the third cycle, hypothermia and tilting plane tests on each rat after IP injection of ethanol 2.5 g/kg showed no tolerance in ANA rats, but the AA rats had clear functional tolerance that was lost after 25 days' abstinence.

Over the next 105 days the AA rats were gradually brought back to free-feeding weight by increasing the number of pellets available. Ethanol consumption gradually fell and that of water rose. This was not due to altered volume of distribution.

Thus, AA rats met most of the criteria for an animal model of alcoholism, but the caloric value of ethanol perhaps contributed to its reinforcing properties. ANA rats drank less than they could metabolize, and aversive effects may have set the upper limit.

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The Effect of DL-Propranolol and its Enantiomers on Ethanol Induced Cardiac Hypertrophy in the Rat

D. King and M. Hirst

Severe subacute intoxication with ethanol causes cardiac hypertrophy in the rat and myocardial disruption is evident on histological examination. The relative weight of hearts (heart weight/body weight) increases by some 10% after 48 hours and by 20% after 96 hours of treatment. Both dry heart weight and cardiac tissue protein increase equivalently. In parallel with the cardiac hypertrophy there is a marked rise in excreted norepinephrine and epinephrine. As beta-adrenoceptor agonists have a trophic influence on the heart it was postulated that the effect of ethanol is caused indirectly through an increased peripheral sympathetic tone. To test this hypothesis rats were given ethanol (10% w/v), with Liquidiet (Bio-Serv), intragastrically, at 8 hour intervals over 48 hours. Pair matched control animals received isocaloric maltose-dextrin and Liquidiet. Other groups of animals (n=6) received dl-propranolol (10, 20 mg/kg), l-propranolol (5, 10, 20 mg/kg), or d-propranolol (10, 20 mg/kg) in saline (1 mL/kg), or saline (1 mL/kg), subcutaneously, at the times of the intubations. The l- and d-propranolols were included to distinguish effects of beta-adrenoceptor blockade from stabilization of cardiac membranes. Following anesthesia with pentobarbital, rats were decapitated, their hearts removed and rinsed with cold water. They were blotted dry after trimming off fat, the aorta and pericardium, and weighed. Data of relative heart weights in the different treatment groups were analysed by ANOVA, with post hoc comparisons of means, with $p < .01$ being considered significant. Both doses of dl-propranolol suppressed ethanol-induced cardiomegaly, whereas neither dose of d-propranolol was effective. l-Propranolol (10, 20 mg/kg) attenuated the cardiac hypertrophy, whereas the lowest dose was ineffective. It is concluded that the rapid development of cardiac hypertrophy in rats severely intoxicated with ethanol occurs, at least in part, through responses mediated by cardiac beta-adrenoceptors.

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Naturalistic Monkey Environment for Studying Social Influences on Alcohol Self-Administration and Alcohol Effects on Social Behavior

T. Crowley and A. Goebel

We have developed techniques for studying high-dose, alcoholic-like drinking among group-living monkeys (Psychoharmacology 92:196, 1987). The preliminary data suggest that high ranking animals assume priority of access to alcohol drinking spouts, causing low ranking animals to avoid the spouts; it appears that low ranking animals thus are "protected" from developing high-dose drinking habits. To more systematically test this hypothesis we wanted several widely separated spouts; this is difficult to arrange indoors. We now are conducting such studies outdoors year-round in a northern climate using a large, naturalistic, outdoor corral. This poster describes our rather inexpensive, but very functional facility, the design of which may make it easier for other investigators to begin using primate social groups in alcohol studies. The Japanese Snow Monkey (Macaca fuscata) ranges further north then any primate except man, adapting to snowy coniferous forests of Northern Japan, where temperatures reach about -5 C. In this species existing laboratory drug studies are complemented by an extensive literature on ethological field studies. M. fuscata adapts well to North American winters, and with some shelter colonies live outdoors in Oregon, Minnesota, Quebec, Michigan, and (unsheltered) in Texas. After consulting Peggy O'Neill at the NIH Poolesville facility, we enclosed a rough ellipse, approximately 32 x 40 m, with a 1 m high chain link fence, surmounted by a 3 m wall of electrically-conductive nylon net. High-voltage brief-pulse charges prevent climbing on or over this net. Materials for this fence cost less than $14.50 per running meter. Weeds and grass grow freely within the ellipse, and seven dead trees, interconnected with ropes, permit climbing and swinging. A hut with post-supported roof but no walls provides sun and rain shelter. A small kennel run, approximately 2.2 x 4.6 m and 1.7 m high, fully roofed over with chain-link fencing, connects the yard with a small, partially-heated building; the monkeys usually have free access between yard and building via the kennel-run, but they may be locked into yard, run, or building. Inside the building are three pens, each approximately 2.8 x 1.5 x 2.2 m. The monkeys usually pass freely among these pens, but we can close doors to separate them. A raceway through this pen area can be divided into separate compartments to isolate animals briefly; the raceway incorporates a squeeze cage, used for administering medications, drawing blood, or weighing animals. Water is continually available in the building. Monkey-chow biscuit rations are given outdoors. Three mouth-activated drinkometer spouts, located about 18.5 m apart in the yard, supply alcohol test solutions for self-administration studies. An observation hut atop the building provides a full view of the yard and houses counters displaying cumulative numbers of 2 ml doses taken at each drinkometer. Using ethologic techniques, one observer records the frequency of occurrence of various social behaviors (e.g., groom, mount, attack, flee). Another observer records self-administration of alcohol doses, by animal and time. In this facility we hope to clarify how pre-existing social relationships influence alcohol consumption, and how alcohol drinking modifies subsequent social behavior. The facility uniquely and unexpensively supports the "psychological well-being of research primates," which now is legally required.

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Symptoms of Intoxication and Hangovers Perceived to Modify Subsequent Alcoholic Beverage Consumption

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The observations that alcoholic patients are significantly less likely than moderate drinkers to have had severe hangovers (Pristach et al., 1983; Harburg et al., 1981; Whitfield et al., 1986) prompted the development of a structured survey of the reasons individuals perceive for drinking and for modifying their drinking practices, including signs and symptoms during and following intoxication. The pilot text of the instrument included anonymous responses from 83 faculty, staff, and medical student volunteers, almost evenly divided as to sex (38 female, 40 male). Ages ranged from 22 to 64 years with most in the 20-30 age group. The signs and symptoms were scaled for intensity or severity. The questions were found to be easy to answer with the extensive pilot version requiring 15 to 60 minutes to complete. The items had face validity and internal cross-checks were answered consistently.

The results of analyses of these pilot data were consistent with preliminary observations and prior studies. More than three fourths (47, 78%) of the drinkers reported decreases in their alcohol consumption over the years, 13 increased, and 10 reported no change. A large variety of influences associated with the decreases were ranked and rated with concerns about driving, increased personal responsibility, nausea, hangovers, and headache cited most frequently.

Hangovers were reported and described by 76%; of these, 75 to 90% reported being influenced to moderate how much or how often they drank an alcoholic beverage for periods of time ranging from months to many years. Thus, the results of the pilot test establishes the feasibility and utility of the instrument.

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Acute Tolerance to Cocaine: Steady State Experiments

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The euphoric and cardiovascular effects of cocaine have been reported to decline more rapidly than the drug leaves the plasma after a single dose. This and other observations suggest that cocaine exhibits acute tolerance development. We reported previously an analysis of single dose cocaine pharmacokinetics and dynamics that modeled apparent tolerance development as an exponential process, describable by a rate constant (and halftime). We have now done steady state experiments to demonstrate tolerance directly and to determine the rate and extent of its development.

Six healthy intravenous cocaine users were studied. They were given an intravenous cocaine regimen consisting of an injection dose followed by a cocaine infusion that exponentially decreased to a constant rate, designed to immediately produce and then maintain a constant cocaine concentration of 750 ng/mL over four hours. Heart rate was measured by computerized continuous electrocardiographic monitor. Subjective effects were evaluated every five minutes by visual analogue scales marking.

Cocaine administration produced a 32±13 BPM (mean±SD) increase in heart rate which reached its peak within 3-5 minutes and began to decline immediately toward a plateau at a level 32±23 percent of the peak response. Approach to the plateau was exponential with a halftime of 38±16 minutes, similar to the value of the tolerance factor we calculated in our single dose studies.

The level of intensity of subjective effects (high, anxiety, and stimulation) had similar contours, developed more slowly, reached a peak after an hour and then declined to baseline at the end of four hours. The halftime for decline in "high" rating was 30±9 minutes.

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The Cocaine Receptor: Reinforcing Potency Correlates with Dopamine Uptake Inhibition

M. Ritz and M. Kuhar

While several binding sites for cocaine have been identified, the biochemical mechanisms mediating cocaine-reinforced behavior in animals and cocaine abuse in humans is unknown. In an attempt to identify the relevant receptors associated with the addictive properties of cocaine, we have compared the published potencies of cocaine and several pharmacologically related compounds in studies of drug reinforced behavior with their binding potencies at both presynaptic uptake sites and postsynaptic monoamine receptor sites.

The affinities of cocaine and related drugs at dopamine and choline uptake sites in striatum, norepinephrine uptake sites in frontal cortex and serotonin uptake sites in the medulla were examined using standard in vitro binding techniques. Inhibition of ligand binding to these sites was determined by analysis of competition curves and calculation of K_i values. Similar values were also determined for dopaminergic, adrenergic and serotonergic postsynaptic sites.

The results of these experiments indicate that drugs which were most potent in drug reinforcement studies were also potent inhibitors of ligand binding at the dopamine, norepinephrine and serotonin uptake sites, but showed little inhibition at the other receptor sites tested. Statistical analyses of the data indicate that inhibition of ^{3}H-mazindol binding to the dopamine uptake site is significantly positively correlated with the reinforcing effects of cocaine-related drugs. Thus, we have shown for the first time that dopamine uptake inhibition is the primary mechanism associated with the reinforcing or addictive properties of cocaine.

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Genetic Factors in Behavioral and Lethal Responses to Cocaine in Rats

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Although the pharmacology and biochemistry of cocaine have been widely studied, little is known about the contribution of genotype in determining response to this increasingly abused drug. The present study was conducted to establish a data base of cocaine-related behavior and to use genetic correlations in examining the biological substrates which mediate behavioral and physiological responses to cocaine. The following rat stocks were used: NBR/NIH, ACI/HSD, F344/CR1BR, LEW/CR1BR and S-D/HSD. Several-fold differences in the efficacy of cocaine in producing locomotor stimulant effects were found, with NBR rats displaying the greatest maximum response, and F344 rats showing the lowest peak locomotor response. Large potency differences were also found, with NBR rats being the most sensitive, while rats from the LEW and F344 strains were the least sensitive. Large differences in lethality in response to cocaine were also seen; 50% of NBR rats tested at 40 mg/kg, and 100% of NBR rats tested at 60 mg/kg died shortly after injection, while no LEW or S-D rats died even at 60 mg/kg. These data should be useful to researchers interested in exploring the mechanisms of behavioral and physiological responses to cocaine.

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Effect of Cocaine on Hippocampal Acetylcholine, Norepinephrine and Serotonin

S. Robinson, K. Hambrecht and E. Pennell

The stimulant amphetamine has been found to increase the activity of cholinergic neurons in the hippocampus by an indirect effect involving noradrenergic neurons (Robinson et al, Naunyn-Schmiedeberg's Arch. Pharmacol. 304:263-269, 1978, and Robinson, Brain Research 397:181-184, 1986). Because cocaine and amphetamine share many behavioral and neurochemical actions, it was decided to study the effect of cocaine on cholinergic neurons, as well as on noradrenergic and serotonergic neurons in the hippocampus. The turnover rate of acetylcholine (TR_{ACH}) was used as a measure of activity of cholinergic neurons and was determined by quantitating the relative incorporation of deuterium label into hippocampal ACh and choline using a mass fragmentographic technique. The activity of noradrenergic and serotonergic neurons was estimated by determining the ratio of metabolites (MOPEG, 5HIAA) to neurotransmitter (NE, 5HT) using HPLC with electrochemical detection. Male Sprague Dawley rats (160 g) were injected with physiological saline or cocaine (30 mg/kg, i.p.). Thirty min after injection and immediately after infusion with phosphoryl [$^2H_9$] choline (15 μmol/kg/min, 9 min, i.v.), rats were killed by focussed microwave radiation (8.5 KW, 1.6 sec). This treatment resulted in a greater than threefold increase in TR_{ACh} ($P< 0.05$) with no significant effect on ACh or choline content. Hippocampal MOPEG content and the ratio MOPEG/NE were significantly increased by cocaine treatment ($P< 0.01$), whereas 5HT and 5HIAA were not significantly affected ($P> 0.05$).

These results suggest that acute cocaine administration increases the activity of cholinergic and noradrenergic neurons projecting to the hippocampus. Additional experiments must be performed to determine if the action of cocaine on cholinergic neurons results from noradrenergic activation. Although hippocampal 5HT neurons are not significantly affected 30 min following i.p. cocaine, it cannot be ruled out that they are affected at other times following such treatment.

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Amantadine Treatment of Cocaine Abuse

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Behavioral pharmacology studies of cocaine suggest that dopamine (DA) agonists attenuate cocaine craving and self-administration. Based on this evidence, an open trial of the indirect DA agonist amantadine was initiated to attenuate cocaine abuse in methadone-maintained patients. Inclusion criteria were male or non-pregnant infertile female methadone-maintained patients, age 21-50, who met DSM-III criteria for cocaine abuse for at least 3 months, confirmed by urine reports. Exclusion criteria were schizophrenia bipolar disorder, epilepsy or current medical illness requiring chronic medication. Amantadine 200 mg po daily was administered for 3 weeks, then the dose was increased to 200 mg po BID for 3 weeks more; ingestion of 200 mg was observed 5 days per week by a nurse. Methadone dosage was unchanged throughout. Self-reports of cocaine craving and use and the Beck Depression Index were obtained before and during treatment once per week; urine drug tests were obtained twice weekly. Twelve patients completed 6 weeks of amantadine treatment: 11 males, 1 female; mean age, 33 (range 23-40); mean duration of cocaine use meeting DSM-III criteria for cocaine abuse, 4.6 yrs (range 1-23). Both cocaine craving and self-reported use declined significantly during amantadine treatment (Wilcoxon matched pairs signed ranks test, $p < .003$ and $p < .004$, respectively). Self-reports of cumulative cocaine use was correlated with urine drug tests positive for cocaine (Spearman $R = .77$, $p < .011$). Initial pretreatment Beck score was related to the degree of decrease in cocaine craving during amantadine treatment ($R = .69$, $p < .022$). Three patients attained and maintained abstinence from cocaine use up to 2 months after treatment. These findings support the need for blind, controlled studies to test the efficacy of amantadine for treatment of cocaine abuse.

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Step-Wise Withdrawal from Cocaine Dependence Outcome of 106 Consecutive Cases

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A step-wise withdrawal procedure utilizing amino acids, amantadine hydrocloride (AH), levodopa (LD), bromocriptine mesylate (BM), and desipramine (DES) was investigated in 106 consecutive subjects who sought medical withdrawal from cocaine dependence. Initial medications given to the 77 subjects in Group I consisted of AH, 100 mg, and tyrosine, 400 mg, given twice per day, and tryptophan, 500 mg, given at bedtime. If, after one week of treatment, the subject reported cocaine craving, continued use, cocaine dreams or the withdrawal symptoms of agitation and depression, BM 2.5 mg or LD 250 mg, given twice per day, was added to the regimen. During the third week of treatment, subjects who could not refrain from cocaine use were administered DES, 25 mg, given three times per day. Group II was comprised of 29 subjects, and they received a higher dosage regimen which consisted of AH, 100 mg, and tyrosine, 400 mg, given four times per day and tryptophan, 1500 mg, at bedtime. When administered, the dosage of LD, was 250 mg, three times per day, BM was 2.5 mg, given two times per day, and DES, 25 mg given three times per day. Group II subjects demonstrated superior outcomes in that more subjects in Group I (20 of 77; 20.6%) than Group II (2 of 29; 6.9%) dropped out of treatment in the first week ($x^2=5.039; P<.05$), and a higher percentage of Group II (25 of 29; 86.2%) than Group I (43 of 77; 61.0%) reported cessation of cocaine use ($x^2=6.350; P<.05$). Although not statistically significant, Group II subjects had slightly longer retention in treatment. and demonstrated more urine conversions from cocaine in urine on admission to absent during treatment.The superior performance of Group II suggests that the dosages and regimen utilized in these subjects should be further studied for medical treatment of cocaine dependence.

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A Comparison of the Effects of Repeated Doses of MDMA (Ecstasy) on Biogenic Amine Levels in Adult and Neonate Rats

J. Rosecrans, S. Robinson, G. Patrick and D. Mokler

MDMA was administered to adult rats as a single 40 mg/kg injection or 40 mg/kg (s.c.) every other day for 4 injections. Sixteen days after the last injection rats were killed rapidly and brain area biogenic amine and metabolite levels determined using HPLC techniques. MDMA produced significant depletions of 5-HT and its metabolite, 5-HIAA, in the hippocampus (Hp) and frontal cortex (FC). 5-HT was depleted to 30% of control values in the Hp following single doses. 5-HT levels were unaffected by MDMA in the hypothalamus, suggesting a differential effect on 5-HT containing neurons. DA levels were significantly increased in the hypothalamus, while frontal cortex NE levels were decreased to 73% of control values following 4 doses of MDMA. These data suggest that MDMA is neurotoxic to 5-HT neurons in the rat.

In a separate study neonate rats were administered MDMA in doses of 10,20 and 40 mg/kg (s.c.) on days 4,6,8 and 10. An analysis of biogenic amine turnover (metabolite/amine ratios) indicated that DA turnover was significantly increased 18 days after the last of 4 doses of either 10 or 20 mg/kg (+18-27% brain), while 40 mg/kg of MDMA induced a decrease in turnover. A similar increase in 5-HT turnover was observed at lower doses (10 and 20 mg/kg); 5-HT levels were also reduced by at least 25% in all MDMA treated rats. In addition there appeared to be a dose-dependent decline in 5-HIAA levels suggestive of an MDMA-neurotoxic effect similar to that observed in the adult.

EFFECTS OF MDMA ON DOPAC AND 5-HIAA LEVELS

DOSE	DOPAC ng/g	5-HIAA ng/g
0	70.6	499
10	85.6 (+21%)	399 (-21%)
20	85.5 (+21%)	361 (-28%)
40	58.9 (-17%)	324 (-36%)

This research was supported by a VCU-Faculty-Grant-in-Aid to JAR.

MDMA (3,4-Methylenedioxymethamphetamine): Selective Neurotoxic Effects and Interactions with Brain Serotonin Systems

G. Battaglia, S. Yeh and E. De Souza

MDMA, a ring-substituted derivative of methamphetamine, has recently received a great deal of attention due to its increasing abuse among certain segments of the population and due to the controversy surrounding its proposed benefits as an adjunct in psychotherapy. MDMA has been reported to possess stimulant properties similar to the amphetamines and psychotomimetic properties somewhat like those of the hallucinogens. Since 5-HT$_2$ serotonin receptors have been postulated to play a role in mediating the hallucinogenic activity of amphetamine derivatives, we examined the relative affinities of MDMA on pre- and post-synaptic brain serotonin recognition sites using radioligand binding techniques. An in vitro pharmacological profile of MDMA at a number of brain recognition sites revealed that MDMA exhibited relatively high affinity (K_i=5μM) for serotonin uptake sites, 5-HT$_2$ and 5-HT$_{1A}$ serotonin receptors. MDMA demonstrated a stereospecificity at serotonin uptake sites (i.e. S(+) more potent than R(-)) consistent with its reported stereospecificity in blocking the active uptake of serotonin and in causing ^{3}H-5-HT release. In contrast, the stereospecificity of MDMA at 5-HT$_2$ serotonin receptors was the opposite of that observed at serotonin uptake sites but consistent with that observed for other hallucinogenic amphetamines. Furthermore, MDMA demonstrated radioligand binding characteristics at 5-HT$_2$ serotonin receptors consistent with those observed for serotonin agonists and similar to those previously reported for other substituted hallucinogenic derivatives of amphetamine. Thus, MDMA may produce some of its mood altering actions by indirect effects at presynaptic serotonin terminals as well as by direct agonist effects at postsynaptic 5-HT$_2$ serotonin receptors. We examined the potential neurotoxic effects of MDMA on monoamine neurons in brain. Short-term (4 day treatment) subcutaneous administration of MDMA in rats produced long-term and widespread neurodegeneration of serotonin axons and terminals in a number of brain regions including the cerebral cortex, striatum, hippocampus and hypothalamus up to 14 days following treatment. Marked, dose-dependent decreases in the content of brain serotonin and 5-hydroxyindoleacetic acid as well as in the density of serotonin uptake sites were observed following MDMA treatment. The neurotoxic effects of MDMA appear to be preferentially localized to serotonergic neurons as there were only minor and inconsistent changes observed in some of the markers for catecholamines. Administration of the selective serotonin uptake blocker citalopram prior to administration of MDMA protected against the neurodegenerative effects of MDMA on brain serotonin in neurons. In vitro autoradiographic visualization of changes in the density of serotonin uptake sites indicated that there may be some morphological selectivity to the neurodegenerative effects of MDMA as little change was observed in regions such as dorsal and median raphe nuclei which contain primarily serotonin cell bodies. We are presently investigating in more detail whether some serotonergic pathways in brain may be more vulnerable than others to the neurotoxic effects of MDMA. In summary, MDMA may exert some of its psychotomimetic effects by both direct and indirect actions on serotonin neurons and short-term administration of MDMA causes profound, widespread and long-lasting neurodegeneration of brain serotonin axons and terminals.

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Identification of 3,4 Methylenedioxyamphetamine (MDA) as a Major Urinary Metabolite of 3,4 Methylenedioxymethamphetamine (MDMA)

R. Fitzgerald, R. Blanke, N. Narasimhachari, R. Glennon
and J. Rosecrans

MDMA ("ecstasy") is a substituted phenylisopropylamine which produces a unique state of enhanced emotional and sensory awareness. The effects of its N-demethylated analog MDA on humans has been characterized as being both LSD-like and amphetamine-like. Both MDMA and MDA have been shown to be fairly selective at destroying serotonin nerve terminals. As a preliminary study to investigate the metabolism of MDMA, male Sprague-Dawley rats were housed in metabolism cages and received a single subcutaneous injection of 40 mg/kg of (+/-) MDMA every other day for eight days. Urine and feces were collected at 24 hour intervals. Plasma was collected on the eighth day four hours after the final dose of MDMA. Initial identification of MDA as a metabolite was suggested by thin layer chromatography of rat urine. MDA and MDMA had the same R_f values as their respective standards when visualized with four different developing solutions. To confirm this observation alkaline urine and plasma extracts were derivatized with trifluoroacetic anhydride (TFAA) and chromatographed using a mass spectrometer as a detector. The mass spectra obtained from the two major peaks of derivatized urine and plasma extracts were identical to authentic TFAA derivatives of MDMA and MDA.

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The Effect of MDA and MDMA ("Ecstasy") Isomers in Combination with Pirenperone on Operant Responding in Mice

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The optical isomers of MDA and MDMA were evaluated as to their ability to disrupt operant behavior (FR-20) in the mouse. These compounds were prepared by acylation of 1-(3,4,-methylenedioxyphenyl)-2- aminopropane with ethyl chloroformate followed by reduction with lithium aluminum hydride and treatment of an etheral solution of the product with HCl gas. The melting points determined after recrystallization agreed with those values obtain by others studying these compounds. All agonists were administered (i.p.) 15 min prior to behavioral testing; animals received daily injections of saline except on test days which were conducted every 3rd day. Results were expressed as ® of vehicle rates of operant responding' vehicle response rates from the day prior to the test session served as control. These compounds as well as S(+)-amphetamine (AMPH) and DOM disrupted behavior in a dose-related manner. ED-50 values indicated the order of potency amongst isomers to be as follows: S(+)-MDA >R(-)MDA>S(+)MDMA >R(-)-MDMA. Both AMPH and DOM were several times more potent than these isomers and disrupted behavior at <1mg/kg. The preadministration of the 5-HT-2 receptor antagonist, pirenperone (PIR), significantly antagonized DOM responding (0.1 mg/kg, s.c., 60 min. prior to 1 mg/kg DOM) supporting previous data obtained in rat drug discrimination studies. In the present studies, pirenperone was observed to antagonize only R(-)-MDA in the mouse operant. The results of this study are in accord with other research conducted in these laboratories. That is, the 5-HT-2 antagonist, PIR, is able to antagonize the effects of R(-)-MDA at a dose comparable to that which blocks the disruptive effects of DOM. On the other hand, this dose of PIR was unable to antagonize the effects of S(+)-MDA or of either optical isomer of MDMA. Apparently, the disruption of behavior produced by the isomers of MDA is via a different mechanism. In addition, the disruptive effects produced by the R(-)-isomer of MDMA appear to be via a mechanism that differs from that implicated for R-(-)-MDA (i.e., a 5-HT-2 mechanism).

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Survey of Medical Advice about Caffeine

J. Hughes, G. Amori, J. Lewis, F. Lavigne and
D. Hatsukami

Scientific reviews differ in whether the use of caffeine is harmful. To determine consensus on the harmful effects of caffeine we asked 784 medical specialists when they advised reduction or abstinence from caffeine. Specialists in private practice, academia, or in both were surveyed. 388 (56%) of physicians responded.

For most conditions, responses were similar across specialties and types of practice. More than 75% of the specialists recommended reduction in caffeine with the following conditions: anxiety, arrhythmias, esophagitis/hiatal hernia, fibrocystic disease, insomnia, palpitations and tachycardia. 50 to 75% of the specialists recommended reduction with hyperactivity, depression, polyuria, and ulcers. Less than 50% recommended reduction with acne, akathesia, depression, dermatitis, diabetes, diarrhea, eczema, glaucoma, headaches, hypothyroidism, irritable bowel syndrome, nocturnal enuresis, pancreatic cancer, post-myocardial infarction, psoriasis, psychosis, restless legs syndrome, scotoma, tinnitus and ulcerative colitis.

Our results indicate a consensus that caffeine is harmful in several common medical disorders. If caffeine is shown to produce behavioral or physical dependence, then our results indicate that caffeine cannot be dismissed as a drug of dependence because it is not harmful.

AUTHORS

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Effects of Nicotine and Nicotinic Agonists on Calcium Influx into Brain Synaptosomes

C. Hillard

The CNS nicotinic cholinergic receptor has been shown to modulate the release of norepinephrine, dopamine and acetylcholine. It is hypothesized that neurotransmitter release results from increased intracellular calcium levels. The experiments carried out in this study demonstrate that nicotinic agonists modulate calcium entry into synaptosomes and characterize this effect. Synaptosomes were prepared from rat forebrain and aliquots (approximately 0.5-0.7 mg of protein) were preincubated in incubation buffer (136 mM NaCl, 5 mM KCl, 0.01 mM $CaCl_2$, 1.3 mM $MgCl_2$, 10 mM glucose and 20 mM Tris, pH 7.65) for 10 min at 30°C. Agonists were added in buffer that also contained 1.0 x10^6 dpm of ^{45}Ca and the incubation was continued for 5 sec. Basal influx was typically between 1.75 and 3.75 pmol ^{45}Ca/mg protein and was diminished in the presence of unlabelled calcium. Influx in the presence of 35 mM potassium was increased by 300-400%. Nicotine produced a concentration-related change in ^{45}Ca influx into synaptosomes, increasing influx at concentrations between 1 and 300 μM. Nicotine did not increase ^{45}Ca influx when the incubation temperature was 4°C or in incubation buffer lacking sodium. The stimulation produced by nicotine was blocked by the nicotinic receptor antagonist mecamylamine and did not occur in the presence of cobalt. The effects of three other nicotinic agonists on ^{45}Ca influx were determined. 1,1-Dimethyl-4-phenylpiperazinium iodide (DMPP) was the most potent and the most efficacious of the agonists investigated, producing a maximal increase at a concentration of 1 μM. Cytisine was also an effective agonist, maximally increasing ^{45}Ca influx at 10 μM. Carbachol was very similar to nicotine, both of which increased ^{45}Ca entry by 20% over control levels at concentrations of 100 and 300 μM. All of the agonists had biphasic concentration response relationships in that the amount of stimulation decreased at high concentrations. These studies offer direct evidence that the result of nicotinic receptor stimulation in the CNS is an increase in the influx of calcium into nerve terminals and support the hypothesis that nicotinic receptor induced release of neurotransmitters results from an increase in the intrasynaptosomal calcium concentration.

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Role of Opioid Mechanisms in the Behavioral Effects of Nicotine

S. Herling and W. Corrigall

The effects of nicotine, heroin, mecamylamine, and naltrexone, administered alone or in combination, were studied in rats trained to respond under a fixed-interval 3-minute schedule of food presentation. Dose-response curves were determined for each drug by administering cumulative subcutaneous doses of a drug 20 minutes prior to the start of a fixed-interval component which consisted of five consecutive fixed-intervals. Daily sessions consisted of three or four separate fixed-interval components, each preceded by a 20 minute time-out. Increasing doses of nicotine (0.1 - 3.0 mg/kg) first increased, then decreased response rates. In contrast to nicotine, heroin (0.03 - 0.6 mg/kg) produced only dose-related response rate decreases. Mecamylamine (0.1 - 3.0 mg/kg) and naltrexone (0.3 - 10.0 mg/kg), when administered alone, had little effect on response rates. However, when administered in combination with increasing doses of nicotine, mecamylamine (1.0 mg/kg) blocked the response rate-increasing effects of 0.3 and 1.0 mg/kg nicotine and partially reversed the rate-decreasing effects of 3.0 mg/kg nicotine. In contrast, the combination of naltrexone and nicotine, at doses of each that alone had no effect on or increased response rates, severely decreased responding. In addition, these combinations of naltrexone and nicotine produced profuse salivation in all rats, an effect not observed when either drug was administered alone. These latter observations may be related to previous results which have suggested a role for endogenous opioids in mediating certain of the behavioral effects of nicotine.

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Effect of Tobacco Withdrawal on Neurohormonal Function

J. Hughes, G. Amori, F. Stewart, G. Arana and R. Workman

Ten smokers first underwent a dexamethasone suppression test (DST) during ad-lib smoking. Cortisols were drawn and dexamethasone (1 mg) given at 2300 on day 1. On day 2, cortisol and dexamethasone levels were drawn at 0800 and 2300. Two weeks later, subjects quit smoking and the DST was repeated as before on the second day of abstinence. Abstinence from tobacco was verified biochemically in nine subjects.

Abstinence increased pre-dexamethasone cortisol levels (drawn at 2300) in all nine subjects, mean = 4.1 and 6.9 ug/dl ($p<.05$). Abstinence did not change the cortisol response to dexamethasone nor the metabolism of dexamethasone.

These results suggest abstinence from tobacco produces a nonspecific increase in cortisol but does not influence the responsivity of the neurohormonal axis. They also suggest that temporary abstinence from smoking does not cause false positive DST results.

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Replacement of Cigarette Smoking with Nicotine Gum: Effects on Information Processing

F. Synder, F. Davis and J. Henningfield

Results from a cigarette abstinence study conducted in our laboratory indicated that conventional measures of cognitive performance declined within 24 hours of tobacco deprivation and remained below baseline levels for the entire 10-day abstinence period. These findings confirm clinical observations that performance impairment may be a determinant of relapse to cigarette smoking. In particular, measures of attention, "working" memory, and arithmetic were disrupted during tobacco withdrawal. The present study examined the efficacy of nicotine gum in reducing the deleterious effects of cigarette abstinence on our computerized Performance Assessment Battery (PAB).

Eight male smokers, who smoked at least one pack of cigarettes per day, participated in the study. Subjects were trained on a variation of the PAB, an approximately 20 minute battery which measures memory, attention and logical reasoning, until stable baseline levels of performance were reached (M = 13 sessions). The study design consisted of 3 consecutive days during which subjects were not permitted to smoke but were given a piece of gum to chew every hour for 12 hours each day. The gum contained either 0, 2 or 4 mg of nicotine but was constant for the 3-day block. Each subject was tested in 3 such blocks at each dose condition. Blocks were separated by 4 days of free smoking.

Irrespective of dose of nicotine gum, PAB performance was best under the cigarette smoking condition. However, PAB performance during the 4 mg dose condition closely approximated the cigarette smoking condition, while the performance data from the 3 days of placebo gum indicated a nicotine withdrawal. Performance during the 2 mg nicotine gum condition was better than that at placebo and worse than that at 4 mg.

These results confirm the viability of nicotine replacement in blocking the performance impairing effects which may be produced by tobacco withdrawal. They also suggest that, at least for heavier smokers, higher maintenance doses of nicotine gum may be required than those readily available with the 2 mg preparation which is available in the United States.

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After Chronic Nicotine or Diazepam, the Intensity of the Anxiogenic Effect of a GABA Depleter is Increased

C. Harris and H. Lal

Dependence upon various drugs causes anxiety as a withdrawal symptom. Pentylenetetrazol (PTZ) discrimination, an animal model of anxiety (Lal and Emmett-Oglesby, Neuropharmacology 22:1423-1444, 1983), has been utilized as a behavioral assay for this subjective effect of withdrawal from nicotine (Harris et al., Psychopharmacology 90:85-89, 1986) and from diazepam (Emmett-Oglesby et al., Eur. J. Pharmacology 92:127-130, 1983). The purpose of this study was to determine the effect of chronic treatment with nicotine or diazepam upon the subjective response to perturbations of the GABA system. Isoniazid was utilized to deplete brain GABA, and diazepam to facilitate GABA activity. Rats were first trained to press levers for food reward in a two-lever operant task. To train the discrimination, they were reinforced for presses on one lever after PTZ (20 mg/kg) and on the other lever after saline. Training was then suspended, and rats were assigned to one of three groups. One group received nicotine, 1.9 mg/kg the first day and 3.75 mg/kg/day thereafter for 15 to 21 days. A second group received diazepam, 20 to 160 mg/kg/day, for 16 to 24 days. The third group received no chronic treatment. After the end of chronic treatment, the rats were tested for the occurrence of a PTZ-like withdrawal stimulus. When this withdrawal effect became minimal, the rats were further tested for response to isoniazid and to diazepam. Isoniazid, 100 and 200 mg/kg, produced a dose-dependent PTZ-like stimulus of low intensity in the control group, and of high intensity in the groups which been given chronic nicotine or diazepam. Diazepam reduced the percentage of rats selecting the PTZ-lever after PTZ, after isoniazid and during withdrawal from nicotine or diazepam. These data indicate that chronic treatment with agents from two diverse classes of dependence-producing drugs increased sensitivity to a drug which lowers brain GABA function. We suggest that this change in sensitivity may be caused by decreased GABA transmission (due to alteration of the GABA-benzodiazepine-receptor/-chloride-ionophore-complex) or increased activity of an anxiogenic endocoid during withdrawal. (Supported by NIDA Grant DA03521)

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Diazepam Effects on the Spontaneous Electroencephalogram (EEG), Evoked Potentials, and Performance in Humans

W. Pickworth and R. Herning

Diazepam is a commonly used drug with abuse potential. The purpose of this study was to quantify EEG changes in humans given diazepam in a wide range of doses. Nine male residential volunteers with a mean age of 28.4 yrs (22-40) and a mean weight of 78.0 kg (63.6-100.0) were given (0, 2.5, 5.0, 10, 20, and 40 mg) of diazepam by mouth in a randomly assigned order of a double blind crossover study. Study days were separated by at least 72 hours. Prior to the drug and at 2, 6 and 24 hrs following the drug, the subjects were comfortably seated in a dimly-lit sound attenuated recording chamber. Two min of spontaneous EEG was recorded while the subject relaxed with eyes closed (EC) and eyes open (EO). Cognitive evoked potentials were illicited by means of the auditory oddball task.

Diazepam had significant effects on several measures of the spontaneous EEG. Diazepam increased power in the beta (14-25 Hz) frequency band and decreased power in the alpha (8-13 Hz) band. Only the diazepam 40 mg increased power in the delta (0.5-4 Hz) band. The mean frequency in the beta band decreased over dose; whereas, alpha frequency increased. The 40 mg dose decreased the delta frequency. The effects occurred in subjects with EC or EO and were orderly across the time periods. Regardless of the dose, the spontaneous EEG in all measures returned to control leads after 24 hrs. Error rate increased in the cognitive task where there were concomitant dose-related decreases in P300 amplitude and time related increases in latency. These results indicate that diazepam has effects on several neuroanatomically and functionally distinct systems. The decrease in attention suggested by changes in the spontaneous EEG and decrements in processing indicated by changes in evoked potentials point to several mechanisms to account for the diazepam-induced performance disruption we observed.

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Differentiation of High Versus Low Dose Chronic Benzodiazepine Dependence in the Rat

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It has long been known that termination of chronic supra-therapeutic dose benzodiazepine (BNZ) treatment can induce dependence manifest as a severe withdrawal (WD) syndrome (Hollister et al, Psychopharm 2:63, 1961). More recent evidence reveals that BNZ dependence can also be induced by long-term therapeutic exposures. The WD signs following chronic high or low therapeutic dose exposure are similar. However, meaningful comparisons between high and therapeutic dose dependencies are lacking. Accordingly, we compared spontaneous WD syndromes obtained following chronic high (HD) versus low dose (LD) chlordiazepoxide (CDP) treatments in the rat. Male S-D rats (300-500 gm) were dosed p.o. bid for 5 wks with fixed doses of 0, 5, 10, 20, 40, 75 or 150 mg/kg CDP or by chronically equivalent maximally tolerable dosing (JPET 226, 100, 1983). At the end of treatment, 20 motor, autonomic and behavioral WD signs were observed daily by experienced raters. The sum of all individual WD sign intensities for each animal at any time point, termed the WD SCORE, was calculated. Chronic steady state blood levels of CDP, norCDP and demoxepam were measured by HPLC (Fed. Proc. 40: 2350, 1981) 4 hours after the last dose in separate groups. All chronic doses gave statistically significant WD compared to controls. The LDR curves for WD Score and the maximum weight loss during WD were both biphasic: the HD component (40, 75, 150, CEMT) was steeper than the LD component (5, 10, 20, 40). To increase the sensitivity for detecting differences between HD and LD dependencies the sign data from the LD groups was pooled and compared to pooled data from HD groups. Ten WD signs were common to HD and LD groups. Of these, 7 were statistically significantly more intense in the HD group than in the LD group. Two WD signs were only seen in the HD group. Chronic blood levels for all components increased linearly with increasing dose in a monophasic fashion. These results differentiate LD versus HD chronic BNZ dependence in an animal model. The biphasicity of the LDR curves for spontaneous WD Score and weight loss suggest of two mechanisms. The absence of biphasicity in the final steady state blood level curves implies it is pharmacodynamic (CNS specific) rather than pharmacokinetic (not CNS specific). The two neural mechanisms of chronic BNZ dependence might be due to different receptor subtypes, conformations of the receptor or receptor coupled counter-adaptive changes.

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Discriminative Stimulus Produced by Diazepam Withdrawal is Increased by GABA Antagonists

S. Idemudia and H. Lal

Withdrawal from diazepam produces anxiety (Petursson and Lader, Br. Med. J., 283:643, 1981). This subjective effect can be experimentally investigated by comparing it with the interoceptive discriminative stimulus produced by the anxiogenic drug pentylenetetrazol (Lal and Emmett-Oglesby, Neuropharmacol., 22:1422, 1983). The present experiments were conducted to determine whether the pentylenetetrazol-like interoceptive stimulus produced by diazepam withdrawal (Emmett-Oglesby, et al., Eur. J. Pharmacol, 92:127-130, 1983) would be altered by drugs acting at the GABA-BZ-picrotoxin modulated chloride ionophore receptor complex. Rats were trained in a two-choice operant task to obtain food reward by selecting one lever after an injection of pentylenetetrazol (PTZ; 20 mg/kg) or the other lever after saline (1 ml/kg). After rats had learned to discriminate PTZ from saline, all of them selected the saline-appropriate lever following saline, Ro 15-1788, or diazepam, whereas some of them selected the PTZ-appropriate lever following bicuculline, yohimbine, picrotoxinin, or ED50 of PTZ. Diazepam (120 mg/kg) was then administered to the rats in a liquid diet twice daily for 3 days so that during spontaneous withdrawal, only 30% of the rats selected the PTZ-appropriate lever following saline. In contrast, all of them selected the PTZ-appropriate lever following Ro 15-1788, bicuculline, yohimbine, picrotoxinin, or the ED50 of PTZ. These data show that chronic diazepam produces a PTZ-like stimulus which is enhanced by antiGABA drugs. Therefore, these data support the hypothesis that the PTZ-like IDS produced by withdrawal from chronic diazepam may be due to a decrease in GABAergic neurotransmission.

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The Effects of Yohimbine on Triazolam Self-Administration in Subjects with Histories of Sedative Abuse

J. Roache, R. Meisch, J. Henningfield and J. Jaffe

The effects of yohimbine (YOH) pretreatment on triazolam (TZ) self-administration were examined in 3 single-blind paid male volunteers with sedative abuse histories who were living on a research ward and were not dependent at the time of the study. Five days each week and over a 3 hr period beginning at 0900 hr, subjects could self-administer a maximum of 18 color-coded placebo or TZ capsules on a signaled concurrent choice 10 min T.O. schedule of reinforcement; with this schedule, a desk lamp was turned on to signal capsule availability and was turned off for 10 min after subjects' verbally requested and ingested their preferred capsule color.

All subjects showed a consistent preference for TZ capsules and showed a general stability in the number of TZ capsules self-administered (at 0.125 mg/capsule) over a 10 day baseline period; the number of capsules self-administered was inversely related to the subjects' initial sensitivity to the psychomotor impairment produced by test doses of TZ administered in an initial phase of the experiment. In two subjects, varying doses of TZ (0.0312-0.25 mg/capsule) were tested and the number of capsules self-administered was inversely related to TZ dose. After varying doses of TZ were tested, all subjects received varying doses of YOH pretreatment (20-40 mg) or placebo in 5 white capsules at 0815 hr. In all 3 subjects, YOH increased blood pressure and heart rate and in two subjects, YOH increased subject ratings of tension/anxiety and decreased ratings of calm/relaxed. With all three subjects, YOH increased the number of TZ capsules self-administered although this effect was not consistently related to YOH dose.

Although preliminary, the current results suggest this self-administration paradigm can show relatively stable dose-related TZ self-administration behavior in subjects with histories of sedative abuse. Also preliminary are the findings that YOH produced a cardiovascular acceleration and increased TZ self-administration and in some subjects produced anxiety-like subjective effects. Further research in this area may provide insights relating to the "self-medication" or "need" hypotheses of drug dependence and may lead to better laboratory analogs of drug-taking behavior.

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The Abuse Potential of Adinazolam: A Comparison with Diazepam, Lorazepam and Placebo

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The subjective effects of adinazolam (ADZ 30 and 50 mg), a triazolobenzodiazepine with clinical specificity thought to be similar to alprazolam, were compared to lorazepam (LZ 2 and 4 mg) and diazepam (DZ 20 mg) in a double-blind placebo-controlled study in recreational sedative drug users. The dependent measures were the Addiction Research Criteria Inventory (ARCI), analog "street value", "use again", "physical high", and "mental high" ratings.

A six treatment cross-over design was used to assess drug effects in groups of five subjects each. Each session was run in a setting intended to create a living room like atmosphere. The ARCI was administered every hour starting at baseline and ending at six hours post dosing. Analog ratings were collected at one and two hours post treatment. All data was statistically analyzed using analysis of variance covarying for baseline ratings (ANCOVA) and Newman-Keuls post hoc multiple comparisons.

The ANCOVA/Newman Keuls analyses of time of onset and magnitude of peak effect discriminated between the mood effects of each active treatment. Using our revised scoring procedure for the ARCI, significant main drug effects were obtained for measures of "mental" and "physical sedation"; each treatment had a mean magnitude of effect significantly greater than placebo on at least one time point. Interestingly, in examining the maximum sedating effects of each treatment ADZ 50 mg produced more "mental" and "physical sedation" than all other treatments. Lorazepam 2 and 4 mg, on the other hand, were the least sedating of all treatments and, in fact, were more "physically stimulating" than diazepam or placebo.

The "mental unpleasantness" measure provided useful data regarding the abuse liability of ADZ. At three hours post dosing (the time of peak onset), ADZ 30 mg produced significantly greater "mental unpleasantness" than placebo. The results of ADZ 50 mg are even more striking. At all times except 4 hours post, ADZ 50 mg produced significantly more "mental unpleasantness" than placebo. However, it should be noted that ADZ 30 mg and ADZ 50 mg, as with all active treatments, resulted in ratings of "physical" and "mental highness" which were significantly higher than placebo, and ADZ 50 mg received ratings of "street value" significantly greater than all treatments except for DZ.

Taken together, these data suggest that DZ has the greatest abuse liability. While LZ and ADZ produces pleasurable effects, subjects' rating of sedation and negative subjective effects indicate that the overall potential for abuse of these drugs may be relatively limited.

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Stereoselectivity of 11-OH-Delta-8-Dimethylheptyl Tetrahydrocannabinol in the Mouse and Dog

P. Little, D. Compton, R. Mechoulam and B. Martin

(-)-Δ^9-THC, the major psychoactive constituent of marijuana, produces a variety of effects in animals and in man, however, the mechanism(s) involved in mediating these actions have not been elucidated. The pharmacological effects of Δ^9-THC may be due to membrane perturbation and evidence for this focuses on the lipophilicity of the cannabinoids. On the other hand, there is evidence which suggests the existence of specific sites of action for the cannabinoids. These interactions may involve specific neurotransmitter receptors, enzymes, plasma membrane constituents or a specific cannabinoid receptor. Evidence for a specific cannabinoid receptor is based on the structure-activity relationship established for numerous cannabinoid analogs (Razdan, 1986), the demonstration of stereoselectivity (Dewey *et al.*, 1981) and the demonstration of specific, saturable binding in brain homogenates (Nye *et al.*,1985). The degree of stereoselectivity demonstrated with the cannabinoids is modest. The synthetic (+)-isomer of Δ^9 and Δ^8-THC have been reported to be 5-100 times less potent than the (-)-isomers (Dewey *et al.*, 1981). The stereoisomers of 11-OH-Δ^8-dimethylheptyl-THC (11-OH-DMH) have been synthesized and tested for their analgesic, anti-convulsant and discriminative properties (Mechoulam *et al.* in press). These isomers were examined for their effects on spontaneous activity, rectal temperature, tail-flick, catalepsy, and the dog static-ataxia model. The (-)-isomer decreased spontaneous activity [4 μg/kg (1-14); ED_{50}(confidence limits)], lowered rectal temperature by 3°C at 21 μg/kg, was analgetic [9 μg/kg (2-39)], and cataleptogenic [19 μg/kg (3-111) following iv administration. In contrast, no ED_{50} values could be obtained with the (+)- isomer in mice up to doses of 30 mg/kg iv. Similarly, in the dog static-ataxia model the (-)-isomer produced signs of static ataxia between 3-10 μg/kg iv, while the (+)-isomer was inactive at 1 mg/kg iv. Therefore the (-)-isomer of 11-OH-DMH was at least 300 times more potent than the (+)-isomer in all tests. This is in contrast to the degree of stereoselectivity reported with Δ^9- or Δ^8-THC. The stereoselectivity seen in the mouse and dog with the isomers of 11-OH-DMH is indicative of what would be expected if a specific receptor interaction was responsible for mediating the action of the cannabinoids. The stereoisomers of 11-OH-DMH may be useful tools in further elucidation of the mechanism(s) of action of the cannabinoids.

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Serum Cannabinoids in Pregnant Rat and Fetus Following Acute and Chronic Administration of Tetrahydrocannabinol (THC)

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Measurement of THC and its metabolite, 11-nor-9-carboxy THC (THC-COOH) in sera of pregnant rats (Long-Evans) and fetuses were carried out following administration of THC (50 mg/kg) by intubation, on gestation day 20 or on day 20 after daily intubation on gestation days 16 to 20 (n = 8/group). Tail tip blood samples were obtained at 1, 2, 4 and 6 hr. after intubation in the acute study and at 1 hr. after intubation on day 20 in the chronic study. Chronically treated rats were sacrificed after sampling. Fetuses were then removed and blood samples obtained. THC and THC-COOH were measured by radioimmunoassay (RIA) utilizing kits provided by the Research Triangle Institute. Since the concentration of these cannabinoids was high, serum samples were diluted with normal rat serum. In the acute study serum cannabinoid levels were as follows:

	Time (hr) After Administration			
	1	2	4	6
THC (ng/ml)	1687 ± 448	1612 ± 410	1420 ± 360	1424 ± 297
THC-COOH* (ng/ml)	275 ± 40	544 ± 98	821 ± 122	1266 ± 225

*Serum THC-COOH levels were significantly different over time ($P < .01$). Trend analysis showed a linear trend between serum THC-COOH and time ($P < 0.01$).

In the chronic study, no significant differences were seen between maternal and fetal serum THC (2270 ± 930 vs 1467 ± 370 ng/ml) and THC-COOH (1056 ± 317 vs 1004 ± 236 ng/ml). Absence of significant differences in maternal and fetal serum cannabinoids is in contrast to previously published studies. Difference in dosages and routes of administration and method of analysis might account for these discrepancies.

Summary: 1. High serum levels (~1500 ng/ml) were achieved 1 hr. following a single intubation of 50 mg/kg of THC to rats on day 20 of pregnancy. This high level was maintained for the 6 hr. studied. THC-COOH showed a linear increase during the same 6 hr. period. 2. Following daily intubation of 50 mg/kg of THC from day 16 to 20 of pregnancy, high levels of THC and THC-COOH were observed in maternal and fetal serum and no differences were seen between the maternal and fetal serum levels for these cannabinoids. Supported by NIDA Grant #04148.

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High Concentrations of Naloxone Lower Natural Killer (NK) Activity

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Several studies performed by other workers have provided some evidence that T lymphocytes may possess opiate receptors (or specific binding sites).

We have hypothesized that naloxone, a specific opioid antagonist, may alter natural killer cell (NK) cytotoxicity activity in normal healthy control subjects, heroin abusers, and methadone maintained patients (MMP). In this study we have incubated peripheral blood mononuclear cells with a wide concentration range (10^{-12} - 10^{-3}M) of naloxone (-), the active enantiomer of naloxone, (which displaces opioids from opiate receptors) and naloxone (+) the inactive enantiomer, (which does not displace opioids from opiate receptor binding sites), in paired experiments, and then have determined NK activity using a standard chromium release assay [1,2].

Our results suggest that both naloxone (-) and naloxone (+) have the same effect on the NK cytotoxicity activity. A reduction of NK cytotoxicity activity is observed when either enantiomer of naloxone is present in concentrations equal to or greater than $1\text{x}10^{-4}$M. This reduction of NK activity is significant (p 0.05) at naloxone (-) concentrations of $7\text{x}10^{-4}$M and above, and essentially zero NK activity observed with concentrations of $1\text{x}10^{-3}$ and above. No alterations in NK activity were observed when naloxone (+) or (-) were present in lower concentrations (10^{-12} - 10^{-5}M).

We conclude that naloxone alters NK activity when present in high concentrations, and that the concentrations of naloxone (-) and naloxone (+) required to effect this reduction in NK activity are similar. This action of naloxone on NK activity does not seem to be mediated through binding to specific opiate receptors on NK cells, because the opiate receptor inactive naloxone is as effective as is the opiate receptor active form of naloxone in this effect.

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Heterosexual Partners of IV Drug Abusers: Implications for the Next Spread of the AIDS Epidemic

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Intravenous drug abusers (IVDAs) represent the group which is at second highest risk for the contraction of AIDS and are predicted as being the major mechanism by which the HIV virus may be spread to the general heterosexual community. Although much has been reported with regards to the at-risk for AIDS needle sharing practices of IVDAs, knowledge about the sexual behavior patterns and practices of the IVDA is scarce. The purpose of this study was to examine the risk of exposure of the HIV virus to non-IVDA males and females posed by sexual contact with IVDAs.

The participants consisted of 107 randomly selected clients who consented to be anonymously interviewed with regards to drug use and sexual behavior patterns and practices. The sample was 58% male, 42% female, 54% black, 35% Hispanic, and 11% white and was statistically indistinct from the total A.R.T.C. population of 2100.

Results indicated that males in comparison with females were significantly more likely to report sexual contacts which were non-IVDAs than IVDAs. Similarly, among those most likely to have been exposed to the HIV virus (either by reported needle sharing and/or sexual contact with other intravenous drug abusers), gender was significantly associated with the number of non-IVDA heterosexual contacts. These findings are supportive of other studies which suggest that the route by which the virus may most likely be spread to the general heterosexual community will be via IVDA males with non-IVDA sex partners.

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Treatment of Heroin Addicts with Buprenorphine: Evaluation Over a Three-year Period

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Buprenorphine is a semi-synthetic opioid with agonist/antagonist properties. Its pharmacological properties soon led some to suggest that it could be used in the treatment of heroin addicts (Mello and Mendelson, Science 207:657-659, 1980). We have already published a report on this use of buprenorphine (Reisinger, Drug Alcoh Depend 16:257-262, 1985).

Between 1983 and 1986 we proposed a treatment with buprenorphine sublingual tablets to 200 heroin addicts . 116 (58%) discontinued the treatment after three appointments or less. 84 (42%) followed the treatment : 3 were excluded from the treatment for various reasons; 28 patients out of 84 (33%) followed the treatment regularly without interrupting for a period longer than one month; 56 patients out of 84 (66%) followed the treatment irregularly, interrupting one or more times for longer than one month at a time, during which they resumed taking heroin; 21 patients out of 84 (25%) recovered. The duration of the treatment was between 2 and 24 months. Follow-up ranged from 6 to 24 months. Dosage of buprenorphine was 2 to 4 mg/day at outset and was gradually decreased. First dose was administered no less than 12 hours after last heroin intake.

This clinical experiment permitted us to draw the following conclusions : (a) Physical withdrawal from heroin with buprenorphine is almost asymptomatic; (b) Buprenorphine's antidepressant effects prevent the secondary depressive syndrome following the cessation of heroin use; (c) Buprenorphine does not induce any euphoric effects; (d) Increasing the dosage of buprenorphine above a ceiling-dose does not increase the agonist effects; (e) All of the patients progressively decreased their dosage of buprenorphine without difficulty during treatment; (f) Buprenorphine's antagonist action reduces the effects of subsequent intake of heroin; (g) Buprenorphine can induce withdrawal symptoms when it is taken immediately after heroin; (h) Withdrawal symptoms at the end of the treatment are negligeable.

As the use of buprenorphine has increased, many cases have been observed of drug addicts injecting themselves with crushed tablets. We have been trying to cope with this problem recently by means of a buprenorphine pill in a new form.

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Benzodiazepine Dependence in Mice: Effect of Dose and Duration of Treatment

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The benzodiazepines (Librium, Valium et al.,) are widely used for their sedative-hypnotic, anti-anxiety, anti-convulsant, and muscle-relaxant properties. In high doses, these drugs can clearly cause physical dependence, although dependence laibility following long-term exposure to low therapeutic doses is not well-established.

We recently described a method for producing and quantifying benzodiazepine physical dependence in mice using high concentrations (up to 0.75% by weight incorporated in chow) for eight weeks (J. Pharmacol. Exp. Ther., 237:462-467, 1986). In the current study, we administered diazepam (DZ) in a lower concentration (0.1%) over two, four, or 16 weeks. Following cessation of drug, the two-week group exhibited mild withdrawal signs for two days, but no convulsions were observed. The four-week group also exhibited withdrawal signs for two days, including convulsions in 73% of the animals. The 16-week group exhibited withdrawal signs for 11 days; 100% exhibited convulsions. We conclude that a clear time relationship exists with respect to DZ physical dependence. In a second set of studies, four groups of mice were fed DZ in chow (0.1%, 0.03%, 0.01%, 0%) for four weeks. A dose-dependent withdrawal syndrome was observed, wtih mice in the low treatment group showing minimal withdrawal signs on only one day. This method therefore, appears to be suitable for future studies in which threshold doses will be administered for several months to determine if significant withdrawal signs are observed.

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SYMPOSIA TITLES

Recent Advances in the Management of Pain

Multiple Opioid Receptors

Effects of Alcohol and Drugs on Fetal Development

Psychiatric Aspects of Alcohol and Drug Abuse
Drug and Alcohol Interactions

Biopsychosocial Predictors of Heavy Substance Abuse

Molecular Mechanisms Involved with the Actions of Abused Substances

Self-Administration Models in Alcohol and Drug Abuse

Opioid and Adrenergic Spinal Receptor Systems and Pain Control

C. Stevens and T. Yaksh

INTRODUCTION

Pain, evoked by somatic or visceral stimulation, reflects the activation of afferent systems which make synaptic contact with second order neurons in the spinal cord and subsequently project to the brain. Pharmacologically, it can be demonstrated that transmission through this pathway is subject to modulation at the spinal cord level by local receptors which are associated with the substrates through which this nociceptive information is transmitted. By virtue of this association, direct activation of several of these receptor systems, with receptor selective agonists, can be demonstrated to produce a powerful and selective analgesia. In the following sections, we will consider: 1) the effect of opioid and adrenergic agonists on spinal function particularly as the effects relate to the processing of nociceptive information; 2) the pharmacology of these spinal systems; and 3) recent studies characterizing changes in drug action when these agents are chronically administered to the spinal cord. It should be stressed that while many systems may modulate nociceptive processing, the "analgesic" action of a particular spinal receptor system can only be established in the *in vivo* bioassay where drugs are given into the spinal space of the unanesthetized animal. Here the effects of the agents on the behavioral response of the animal to a noxious stimulus can be assessed. Such studies are accomplished in man by intrathecal and epidural drug administration and were facilitated in animals by the development of a simple model in which the spinal intrathecal space is catheterized (Yaksh and Rudy 1976).

EFFECTS OF RECEPTOR SELECTIVE AGENTS ON SPINAL NOCICEPTIVE PROCESSING

Opioids

In spinally transected animals at relatively low doses, systemically administered opiates reduce: 1) the polysynaptic ventral root reflex (Wikler 1950); 2) the response of dorsal horn neurons otherwise evoked by activity in Aδ/C, but not Aβ fiber primary afferents; and 3) the size of the cutaneous receptive field of dorsal horn neurons induced by a variety of somatic and visceral stimuli which evoke pain behavior (Le Bars *et al.*, 1975; Yaksh 1978a; Einsphar and Piercey 1980). Systemic morphine at analgesic doses in paraplegic man will attenuate the polysynaptic reflex of the tibialis anterior muscle evoked by sural nerve stimulation with no effect on the monosynaptic H reflex (Willer and Bussel 1980). Importantly, in animal studies this inhibitory effect can be demonstrated on spinal populations of nociceptive neurons which project to supraspinal centers (Jurna and Grossman 1976; Jurna and Heinz 1979). By other criteria, the effects of systemically administered opiates on the activity of nociceptive neurons in spinal transected animals are mediated by an opioid receptor: a) ordering of the structure-activity relationship (i.e. the ability of different opioids to suppress nociception *in vivo* is in accord with their efficacy on *in vitro* opiate receptor bioassay systems (Iwata and Sakai 1971; Yaksh 1978a; Piercey *et al.*, 1982); and b) the effects are dose-dependently antagonized by opioid antagonists (Yaksh 1978b).

With regard to mechanism of action, focal application of opiates within the substantia gelatinosa in the vicinity of the substantia gelatinosa (Zieglgansberger and Bayerl 1976; Duggan *et al.*, 1981) will suppress the spinal nociceptive neuron activity otherwise evoked by stimuli which emit escape behavior in the unanesthetized animal. Opioid ligand binding studies have demonstrated that following dorsal root lesions, either produced by section or by the afferent neurotoxin, capsaicin, major but subtotal reductions in opioid binding occur in the dorsal horn (LaMotte *et al.*, 1976; Gamse *et al.*, 1979), suggesting a significant proportion of opioid binding sites in the dorsal horn exist on the terminals of small primary afferents. These results suggesting a presynaptic action of opiates are consistent with the ability of opioids to inhibit the release of substance P from small primary afferents in a naloxone reversible fashion (Jessell and Iversen 1977; Yaksh *et al.*, 1980). The incomplete depletion of dorsal horn opiate binding and the ability of opioids to suppress excitatory postsynaptic potentials evoked in dorsal horn neurons by excitatory amino acids (Zieglgansberger and Bayerl 1976), however, suggest an additional action directly on the second order neuron.

Intrathecally or epidurally injected opioids have been shown to result in a powerful analgesia as assessed in a variety of experimental pain models in man and animal and in the clinical models of postoperative and chronic pain (see Yaksh and Noueihed 1985). All nociceptive input may not be equally altered by spinal opiates. Thus, consideration of the types of clinical pain affected, has indicated that following epidural morphine, the characteristic sensation most readily relieved by epidural morphine is that described as continuous and originating from deep somatic and visceral structures. Less predictably managed are intermittent somatic and visceral (intestinal obstruction) pain. Cutaneous (incisional) pain is poorly modified by spinal morphine at "analgesic" concentrations. Neurogenic pain such as associated with deafferentation, is often little modified by even high doses of spinal opiates (Cousins and Mather 1984). This differential effectiveness of spinal opiates against different types of pain demonstrated in man and animals reflects upon the spinal substrates whereby the information from these stimulus modalities are coded and with which the opiate receptor is associated.

In vitro pharmacological and biochemical analysis suggest the existence of several potentially distinct classes of opioid receptors: μ (μ_1/μ_2), δ, κ, ε and σ (see Yaksh 1984). The initial evidence of a functionally selective modulation of spinal nociceptive processing emphasized the role of μ preferring agonists (e.g. morphine). Animal studies have now demonstrated that both δ and κ spinal receptor systems are also functionally able to alter nociceptive processing (Wood *et al.*, 1981; Yaksh 1983). Several lines of evidence support the contention that these several classes of ligands act upon discriminable receptors: a) differential structure-activity relationships for μ-, δ- and κ-agonists on different nociceptive measures (Schmauss and Yaksh 1984); b) distinguishable pA_2 values of naloxone for the receptors acted upon by putative μ, δ and κ receptor agonists (Tung and Yaksh 1982); and c) differential cross-tolerance (Yaksh 1983; Tseng 1982). With regard to the latter point, it has been shown that animals rendered tolerant to a μ agonist (such as morphine) show little loss of response to a δ receptor preferring ligand (e.g. DADL) (Yaksh 1983). It should be stressed that this lack of cross-tolerance is dependent upon the magnitude of morphine exposure. Reductions in the response to other ligands will also occur at higher concentrations (Stevens and Yaksh 1986; Russel *et al.*, 1987, see below).

The principal agents employed in man (such as morphine or meperidine) are μ agents. The δ-preferring peptide, DADL, has also been shown to be a powerful analgesic in man after intrathecal administration (Onofrio and Yaksh 1983; Moulin *et al.*, 1985; Krames *et al.*, 1986), though the selective δ agonist, D-Pen^2-D-Pen^5-enkephalin, has not been examined. β-Endorphin in man produces a powerful analgesia when given spinally (Oyama *et al.*, 1980). Though it may act on several sites, one of which is designated as the ε receptor, systematic animal studies have suggested its effects are mediated by a site similar to that acted upon by morphine (Tung and Yaksh 1982; Yaksh *et al.*, 1982).

Considering the κ site, a number of traditional "partial" agonists, such as nalbuphine or butorphanol, are thought to act in part at the κ site. Both agents in man have been shown to be mildly effective as analgesics following epidural administration (Weintraub and Naulty 1985; Wang *et al.*, 1985). In general, κ receptors in animal models are weakly effective (Schmauss and Yaksh 1984; but see Wood *et al.*, 1981). Whether these agents will be useful under different clinical conditions to produce adequate analgesia remains to be seen. This limited effect appears to occur not necessarily because these agents are "partial agonists", but because the receptor with which they interact (κ) may be less efficiently coupled to spinal systems relevant to pain processing.

Adrenergic receptors

Considerable data indicated that bulbospinal noradrenergic systems would alter spinal nociceptive processing (Anden *et al.*, 1966). Such pathways were shown to be activated by brainstem microinjections of morphine (Yaksh and Rudy 1978). Local iontophoretic administration of α-adrenergic agonists into spinal cord will produce a powerful suppression of spinal nociceptive processing (Headley *et al.*, 1978; Fleetwood-Walker *et al.*, 1985). These observations suggested that spinal adrenergic agonists should produce a comparable modulation. Spinal administration of adrenergic agonists in several species has indeed been shown to yield a powerful analgesia (see Yaksh 1985). The pharmacology of this effect revealed that the phenomenon reflects an action mediated by an α_2-receptor. Thus, the relative activity of spinally administered agonists is: ST-91 (a clonidine analogue) > norepinephrine > methoxamine >> isoproterenol = 0. The effects of clonidine, ST-91 and norepinephrine were antagonized by the selective α_2-antagonist yohimbine (Howe *et al.*, 1983). In man, clonidine, an α_2-agonist, has been usefully administered epidurally in cancer patients for the treatment of pain (Coombs *et al.*, 1984; Tamsen and Gordh 1984).

Drug interactions

The antinociceptive effects of morphine following intracerebral administration will synergize with the effects of spinal morphine (Yeung and Rudy 1980). The apparent synergy is thought to result from the property of the inhibitors. Thus, electrophysiological studies have demonstrated that bulbospinal pathways and spinal opiates (Yaksh 1978a; Gebhart *et al.*, 1984) both serve to decrease the slope of the stimulus intensity-response curve. For spinal neurons, such a decrease in slope constitutes a reduction in the gain of the system. The interaction between two such systems would have a net outcome which might be expressed as the product of the gain reductions and not the sum. Given the role of bulbospinal adrenergic pathways in mediating a portion of the opiate effect at the level of the brainstem, it is reasonable to conclude that intrathecal α agonists might potentiate the effects of intrathecal morphine. Such a synergy has indeed been demonstrated (Monasky and Yaksh 1986; Wilcox *et al.*, 1987). In brief, a near maximum analgesic effect may be generated by combinations of α agonists and morphine at doses which alone produce only minimal effects.

TOLERANCE AND CHRONIC SPINAL DRUG EXPOSURE

As outlined above, it is clear that the selective alteration of the animals' organized response to a noxious somatic or visceral stimulus can be achieved by the spinal administration of opioid and α-adrenergic agents. As indicated, in several instances, these agents have been shown to produce a useful change in the humans' response to acute and chronic pain. The use of spinal receptor selective agents for long term administration, however, has the same caveat as the chronic use of opiates given systemically; repeated or continuous dosing will frequently result in the progressive elevation of the concentration of drug required to produce a given effect, i.e. tolerance. Thus, in man with the chronic spinal administration of morphine, a highly variable increase in drug utilization has been noted with 1 to 30-fold increases being repeated in cancer patients over a 24-week period (Yaksh and Onofrio 1987). Similar results have been reported in more systematic, single center studies (Krames *et al*., 1985; Shetter *et al*., 1986).

To assess the effects of long-term spinal administration, several issues immediately pose themselves regarding the phenomenon of tolerance. First, following chronic spinal administration of receptor selective ligands, is the rate of loss related to the chronic treatment concentrations? Second, are there differences in the rate of tolerance development between different classes of spinal receptors which produce analgesia? Third, within a given class of agents, is the rate of tolerance development altered by the potency of the agent?

We have recently utilized an osmotic minipump coupled to an indwelling lumbar intrathecal catheter to deliver different concentrations of several antinociceptive agents in the unanesthetized rat (Yaksh and Stevens 1986). With this schedule of administration, an equilibrium is reached at the receptor biophase and concentration of drug remains constant throughout the infusion period. This is a marked improvement over bolus injections where peak and trough drug concentrations occur and where different concentrations yield different times of drug-receptor exposure (e.g. increasing dose yields not only a higher agonist concentration but a greater time of receptor exposure). Drug is continuously administered at the rate of 1 μl/h, for 7 days, and the antinociceptive effect is measured by the hot plate test. Testing on day 0 before the implantation of the minipump i.t. catheter assembly and daily thereafter allows an assessment of the time course of tolerance development. Chronic spinal administration of three log-spaced concentrations each of morphine, sufentanil, or DAGO (μ-opioid specific agents), DADL (δ-opioid preferring), or ST-91 (an analog of clonidine, α_2-adrenergic agonist) yields a dose-dependent increase in hot plate latency after 1 day of infusion (Stevens and Yaksh 1987). These hot plate values then decline over time, until by three to five days, they are not different from saline-infused controls, i.e. tolerance has developed. Standardization of the raw hot plate latency to maximum percent effect values and calculation of the area under the time course curve (AUC) yields a quantification of the time needed to return to baseline values for each dose in each treatment group. An index of the rate of tolerance development is obtained by plotting the maximum effect of each infusion dose (obtained on day 1) against the area under the time course curve. These points, for any drug, fall on a straight line. This analysis shows that within each treatment group the *rate* of tolerance development is independent of the dose infused. Furthermore, comparison across treatment groups show that the same rate of tolerance development is obtained for μ, δ and α_2 antinociceptive agents in the spinal infusion model (i.e., the slope of the MPE vs. AUC curves do not differ, see Table 1). This similarity of rate constants suggests the involvement of common mechanisms of tolerance to these several drugs. Given the lack of a symmetrical tolerance between these agents (see below), the tolerance mechanism(s), though common, may be independent. Finally, based on the ED_{50} values observed on the hot plate after intrathecal injections in the rat, the relative potency of the three μ agonists is sufentanil $\geq$ DAGO > morphine. As indicated in Table 1, there is no difference in the rate of tolerance development between these agents when administered by chronic infusion. Thus, simple potency differences do not alter the time course of tolerance development.

TABLE 1. Slope of MPE vs. AUC Curves for the Effect of Agents Chronically Infused in the Rat Spinal Space on the Hot Plate Response

Infusion Drug	Infusion Doses[a]	Slope of MPE vs. AUC Curve
Morphine	2, 6 or 20	0.32 (0.25 - 0.40)[b]
DAGO	0.1, 0.3 or 1.0	0.48 (0.37 - 0.58)
Sufentanil	0.06, 0.2 or 0.6	0.42 (0.34 - 0.51)
DADL	2, 6 or 20	0.43 (0.29 - 0.57)
ST-91	3, 10 or 30	0.37 (0.23 - 0.50)

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[a] In nmol/h; each dose infused in 4-6 animals.
[b] Slope (95% confidence interval).

CROSS-TOLERANCE

An important issue relates to cross-tolerance between the several classes of agents. Using the above paradigm, animals were infused for seven days with one of three concentrations of morphine or saline, and then each received a single bolus dose of a "probe" drug to allow construction of dose response curves. Table 2 presents the ED_{50} values at different morphine infusion concentrations for morphine, DADL and ST-91 given as the probe drug.

TABLE 2. IT ED_{50} Values[a] for Morphine, DADL and ST-91 Assessed in Rats after 7 Days of Intrathecal Morphine Infusion

Probe Drug	Morphine Infusion Concentration (nmol/μl/hr)			
	0 (Saline)	2	6	20
Morphine	0.7 (0.3-1.9)[b]	3.1 (0.4-24)	39 (20-73)	83 (49-142)
DADL	0.2 (0.1-3)	0.7 (0.2-2)	0.5 (0.1-2)	5.9 (4-8.6)
ST-91	3.0 (0.9-9.4)	27 (16-47)	39 (19-83)	81 (35-188)

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[a] Each dose response curve is constructed from results measured in 12 to 18 animals.
[b] ED_{50} value (95% confidence interval).

These results indicate only minimal signs of cross-tolerance. Thus at the high concentration of morphine (20 nmol/μl/hr), the ED_{50} for morphine increased by a factor of 120. The ratio increase for DADL and ST-91 was 30 and 20, respectively.

Given the above results, we consider that rational strategies to produce longer term utilization of receptor selective drugs may thus theoretically involve several approaches: 1) Given the lack of a symmetrical cross-tolerance between several classes of receptor selective agents, alternating agents such as μ, δ and α_2 might be considered. 2) Employing combinations of agents which have shown synergistic interactions (e.g. opioid and α_2-agonists). As these combinations are able to produce full antinociceptive effect with a minimal degree of receptor occupancy, a less pronounced tolerance may be produced or have a slower time course. 3) Recent studies have suggested that it is possible to manipulate opioid receptor number with antagonists. Thus, the chronic systemic administration of an opiate antagonist, naltrexone, has been shown to double the number of opiate binding sites in rat brain (Zukin and Tempel 1986). These animals are then more sensitive to morphine administration as compared to controls, suggesting that the increased opiate receptors measured are indeed functionally coupled. This approach might theoretically be employed to upregulate opiate receptors in tolerant patients by intermittent opiate antagonist infusions with supplemental analgesia provided by α_2-agonists or local anesthetics.

In conclusion, consideration of opioid and adrenergic agents able to alter the pain message at the spinal level reveals potential therapeutic approaches to pain control. Animal studies must demonstrate basic mechanisms, pharmacokinetics and non-toxicity before a rational and safe clinical trial can be implemented. Finally, tolerance and cross-tolerance studies are important to optimize drug regimen in patients requiring repeated or continuous administration of analgesics for chronic pain states.

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Multiple Opioid Receptors

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Abuse of opioids continues to represent a major medical and social problem. Improved understanding of existing opioid drugs and development of novel agents with reduced dependence potential requires appreciation that the diversity of opioid effects in the human or animal body results from complex actions at multiple types of opioid receptors located at multiple sites in the central nervous system and in the periphery.

A CPDD symposium, chaired by T.F. Burks and A.E. Takemori, explored current concepts and data relating to the functional implications of multiple opioid receptors. Several important new concepts were brought out during the symposium. It is now evident that research attention in a number of laboratories focuses on a restricted number of opioid ligands that are proving their value as probes of functions associated with specific types of opioid receptors. Morphine, the classical prototype opioid that exhibits preference for the mu opioid receptor, is included in many studies as a useful standard of reference. The peptides [D-Ala^2,NMePhe^4,Gly-ol]enkephalin (DAGO) and [MePhe^3,D-Pro^4]morphiceptin (PL017) are more frequently included in investigations as highly selective mu agonist ligands, cyclic [D-Pen^2,D-Pen^5]enkephalin (DPDPE) or cyclic [D-Pen^2,L-Pen^5]enkephalin (DPLPE) as highly selective delta agonists, and U-50,488H as the nonpeptide kappa agonist. Selective antagonists, recently available, are used with increasing frequency. The selective antagonists include β-funaltrexamine (β-FNA) and cyclic D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2 (CTP) for mu receptors, N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (ICI 174,864) for delta receptors, and the very recently introduced nor-binaltorphimine (nor-BNI) for kappa opioid receptors.

It is now possible to begin gaining a coherent picture of functions associated with distinct types of opioid receptors as different investigators, using different techniques and endpoints, present data obtained in individual systems with the same highly selective opioid receptor agonists and antagonists. When different investigators use the same ligands, the similarity of results is striking and confounding variables, such as species differences, are much more readily identified than when totally different, and often nonspecific, ligands are employed by different investigators.

The drugs and peptides employed originally to allow proposals for classification of opioid receptors show distinct preferences for specific types of opioid receptors: morphine for the mu type, ketocyclazocine for the kappa type, N-allylnormetazocine (SKF 10,047) for the sigma type, [D-Ala^2, D-Leu^5]enkephalin for the delta type, and, in some tissues, β-endorphin for the epsilon type. However, these ligands are not sufficiently selective in most cases to allow identification of functions associated with a single type of receptor.

Dr. Brian Cox, Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD, described radioligand binding conditions that allow identification of binding to the three major types of opioid receptors, mu, delta and kappa, by nonselective ligands. For example, complex Scatchard plots obtained from binding of ethylketazocine (EKC) alone can be simplified by the addition of selective ligands to the binding assay with the aim of reducing EKC binding to specific types of receptors. Addition of DAGO eliminates, by competition, binding of EKC to mu receptors, and addition of U-50,488H eliminates kappa binding. Addition of either selective ligand eliminates binding to one type of receptor and results in a more linear Scatchard plot reflecting binding primarily to the unoccupied mu or kappa receptor. DAGO, DPDPE and U-50,488H are of value as selective ligands in radioligand binding studies. Among antagonists, naloxone is useful, displaying a K_D of 1-2 nM at mu receptors, but is only about 10-fold selective. Nor-BNI is selective for kappa receptors, with a K_D of approximately 1 nM. ICI 174,864 has a high K_D of about 200-400 nM, indicating low potency, but is highly selective. Natural ligands tend to be relatively nonselective. The longer dynorphin peptides, such as dynorphin-(1-13), dynorphin-(1-17) and α-neo-endorphin, act mainly at kappa opioid receptors but possess mu activity as well. Shorter dynorphin peptides, such as dynorphin-(1-8), are relatively nonselective. The natural enkephalins possess both delta and mu activity. Similarly, β-endorphin is not selective in binding assays.

An important feature of opioid receptors is their uneven distribution in different tissues. For example, estimates based on B_{max} values indicate that the cortex of guinea pig brain contains approximately 23% mu, 12% delta and 65% kappa receptors. In contrast, rat whole brain contains approximately 47% mu, 34% delta and 19% kappa receptors. Regional differences also occur even within the same species.

There have been recent advances in understanding the regulation of opioid receptors by sodium and by guanine nucleotides. Sodium (IC_{50} 10-20 mM) inhibits agonist binding at mu and delta receptors, with less effect at kappa receptors. The apparent effect of sodium at delta receptors is to decrease the apparent number of opioid agonist binding sites. This effect has been observed at delta receptors in NG108-15 neuroblastoma-glioma hybrid cells and in guinea pig brain membranes. In 7315c pituitary tumor cells, which possess mu opioid receptors, the major effect of sodium seems to be a shift to lower affinity states of mu receptors, with an overall apparent decrease in mu receptor affinity. As relatively low concentrations of sodium can alter agonist binding, an effect in the low-sodium intracellular environment is suggested. Exposure of 7315c or NG108-15 cells to the sodium ionophore, monensin, increased intracellular levels of sodium and decreased mu and delta receptor agonist binding, respectively. These data suggest that sodium acts at intracellular regulatory sites to decrease opioid receptor binding at both mu and delta receptors.

Guanosine triphosphate (GTP) also affects binding of agonists, but not antagonists, in guinea pig cortical membranes and in 7315c cells. Binding of mu and delta agonists is reduced by addition of 0.1 nM of GTP, probably because of a shift to low affinity states of the receptors. Kappa receptor binding is affected less than mu or delta binding by GTP.

In vitro studies designed to examine agonist potencies in initiating opioid receptor-mediated events in brain have revealed striking species differences. Release of norepinephrine evoked by potassium depolarization was measured in slices of guinea pig and rat cerebral cortex. In guinea pig cortical slices, mu, delta and kappa opioid agonists produced concentration-dependent inhibition of potassium-stimulated release of norepinephrine, apparently by actions at presynaptic opioid receptors on terminals of projections from the locus ceruleus. In guinea pig cortical slices, actions of DAGO were blocked by naloxone, U-50,488H was blocked by nor-BNI. Rat cortex, by contrast, is known to contain few kappa opioid receptors. In slices of rat cortex, only mu opioid agonists, not delta or kappa, produced inhibition of potassium-stimulated release of norepinephrine. The same species difference in response to opioids was observed in guinea pig and rat cerebellum.

An interesting aspect of the studies of opioid inhibition of potassium-stimulated release of norepinephrine from guinea pig brain slices was the demonstration of selective opioid tolerance. Guinea pigs were made tolerant to either morphine (mainly a mu agonist) or U-50,488H (kappa agonist) by continuous infusion for 6 days by means of implanted osmotic minipumps. In brain slices from animals infused with morphine, there was a decreased effect with DAGO, whereas U-50,488H was fully active. In brain slices from animals infused with U-50,488H, there was reduced responsiveness to U-50,488H, whereas DAGO was fully active. The tolerance induced by opioid infusion was thus specific for either mu or kappa receptors. These studies have provided evidence for biochemical and functional distinctions between types of opioid receptors.

Dr. A.E. Takemori, Department of Pharmacology, Medical School, University of Minnesota, Minneapolis, discussed use of antagonists to provide pharmacological characterization of different types of opioid receptors. Naloxone pA_2 values can be used to classify different actions of opioid agonists. For example, mixed opioid agonist-antagonist drugs displayed lower naloxone pA_2 values than those obtained with pure agonists. Interestingly, different centrally-mediated opioid endpoints in some cases had different naloxone pA_2 values: analgesia, respiratory effects and body temperature effects.

However, the inferences to be made by use of naloxone to discriminate between types of opioid receptors were limited. Receptor selective antagonists would be expected to provide much more precise information. Portoghese and Takemori theorized that a complementary molecule possessing an electrophile would interact with opioid receptor nucleophilic sites to generate covalent binding if the electrophilic substitution were adequately chemically reactive. After 12 years of effort, β-chlornaltrexamine (β-CNA) was developed. β-CNA interacts irreversibly with opioid receptors as an opioid-selective affinity label, but does not differentiate between types of opioid receptors. They went on to develop β-FNA, a derivative of naltrexone, which has high affinity for mu opioid receptors. Many other analogs have been prepared and examined, but none is more selective than β-FNA for mu antagonism, implying strict spacial requirements of the antagonist receptors.

In *in vitro* preparations of guinea pig ileum, which contain mu and kappa opioid receptors, morphine and EKC display different naloxone pA_2 values. After treatment with β-FNA, the naloxone pA_2 value for EKC is not altered, but morphine acquires the same naloxone pA_2 value as EKC, implying that morphine acts at the EKC (kappa) receptor after blockade of the mu receptor by β-FNA. In *in vitro* preparations of mouse vas deferens, which contain mu, kappa and delta receptors, treatment with β-FNA eliminates mu receptors without altering the naloxone pA_2 for EKC or [Leu]enkephalin, which act primarily at kappa and delta receptors, respectively. In membrane radioligand binding studies, naltrexone and morphine binding are inhibited by β-FNA, whereas enkephalin binding is not significantly altered.

When administered to intact animals, β-FNA behaves as a reversible kappa agonist (as shown in the abdominal stretch test of analgesia) and an irreversible mu antagonist. Even 48 hr after administration of β-FNA, morphine analgesia can be persistently inhibited. β-FNA can block development of morphine dependence in animals if given before morphine and can block ability of morphine to alleviate the withdrawal response if given after development of morphine dependence.

A more recent approach to development of selective opioid antagonists has been the creation of bivalent antagonist ligands with two pharmacophores at the ends of chemical spacer groups. An early compound, TENA, had affinity for the kappa receptor and displayed a kappa/mu ratio of approximately 5. A more recent compound, BNI, is much more selective as an antagonist at kappa opioid receptors and displays a kappa/mu ratio of 137.5. As expected of a kappa antagonist, BNI and nor-BNI block actions of kappa agonists in preparations of rabbit vas deferens, primarily a kappa receptor tissue. In vivo, BNI and nor-BNI block actions of kappa, but not mu or delta, opioid agonists. Nor-BNI has high binding affinity for kappa opioid receptors in radioligand binding assays.

The development of selective receptor antagonists has provided useful pharmacological tool for studies of opioid receptors and has allowed further differentiation of receptor types.
Dr. Thomas F. Burks, Department of Pharmacology, College of Medicine, University of Arizona Health Sciences Center, Tucson, Arizona, described differentiation of receptor specific central and peripheral opioid actions by the use of highly selective opioid agonists and antagonists. In unanesthetized mice, mu agonists such as DAGO and morphine given intracerebroventricularly (i.c.v.) are more potent in inducing analgesia than delta or kappa agonists. To ascertain that mu and delta effects are in fact mediated by pharmacologically distinct receptors, selective receptor antagonists were employed for selective antagonism of mu or delta effects. Prior administration of β-FNA, a mu opioid antagonist, produced a large rightward shift in the dose-response curve for i.c.v. morphine, but did not alter analgesic responses to i.c.v. DPDPE, a delta agonist. β-FNA thus discriminated between mu and delta opioid receptor mediated events. Prior administration of the delta antagonist, ICI 174,864, produced dose-dependent inhibition of analgesic responses to i.c.v. DPDPE, but did not alter responses to i.c.v. morphine. The mu antagonist thus selectively blocked the mu agonist and the delta antagonist selectively blocked the delta agonist. These data indicate that mu and delta agonists act at distinct recognition sites in the brain to induce analgesia.

When given intrathecally (i.t.) and evaluated in the hot plate test of analgesia, the delta opioids were relatively more potent than after i.c.v. administration and were, in fact, close to morphine in analgesic potency.

Morphine and other opioids can act at multiple sites to inhibit gastrointestinal transit. Brain sites responsible for opioid antitransit effects were evaluated in rats and mice by i.c.v. administration of the receptor selective agonists, DAGO, DPDPE, and U-50,488H. Given i.c.v., DAGO (and other mu agonists) produced dose-related inhibition of gastrointestinal transit, but delta and kappa agonists were inactive. However, when given i.t. in mice, both mu and delta agonists produced antitransit effects, but kappa agonists did not. Thus, brain mu receptors, but not delta or kappa, receptors inhibit transit, but in the spinal cord both mu and delta receptors inhibit transit.

Opioid peptides, because they do not readily cross biological membranes, can be of utility in studies to determine sites of opioid actions. Systemic (s.c.) administration of the mu opioid peptide, PL017, produces antitransit effects in mice at doses less than those required to induce analgesia, thus indicating a peripheral mu opioid antitransit site. The peripheral antitransit effects of s.c. given PL017 are blocked by the peptidic selective mu opioid antagonist, CTP, also given s.c. Thus, present data obtained in mice indicate that mu opioid receptors in the brain, spinal cord and periphery are involved in opioid antitransit effects. Delta receptors in the spinal cord, but not brain or periphery, inhibit transit. Kappa receptors appear not to be linked with antitransit activity of opioids.

In most mammalian species, including man and dogs, conventional morphine-like opioids increase contractions of intestinal circular smooth muscle. In ex vivo vascularly perfused segments of dog small intestine, morphine-like opioids given intraarterially (i.a.) induce dose-related, naloxone-sensitive phasic contractions of circular smooth muscle. Both mu and delta selective opioid agonists induce contractions, with DAGO and DPDPE approximately equipotent, but DAGO more efficacious. By contrast to the mu and delta agonists, kappa agonists such as U-50,488H caused little or no contractile activity in the intestinal segments. The contractions induced by mu agonists were relatively more sensitive to blockade by the neurotoxin, tetrodotoxin, than delta agonist-induced contractions, thus indicating that the mu receptors in the wall of the dog intestine are associated mainly with neurons, whereas delta receptors may be associated mainly with smooth muscle.

Experiments with agonist and antagonist opioid drugs that act selectively at only one type of opioid receptor in brain, spinal cord, or periphery, can effectively separate functional aspects of central and peripheral opioid receptors associated with sensory perception, behavior, or visceral regulation.

Dr. Jeffrey Vaught, Janssen Research Foundation/McNeil Pharmaceutical, Spring House, PA, described the use of mice with special genetic traits to explore different types of opioid receptors. This line of research grew out of an interest in determining whether brain delta opioid receptors are inseparably coupled somehow with mu receptors. It had been postulated that thermal analgesia is predominantly a mu event, and that delta opioid receptors might serve mainly to modulate mu receptor mediated analgesia. Several laboratories have found comparable naloxone pA_2 values for mu and delta agonists, therefore, naloxone pA_2 values provide, in themselves, no evidence for differences between mu and delta receptors. However, ICI 174,864 has been found to block DPDPE (delta) analgesia without blocking mu analgesia. Moreover, mu-delta cross-tolerance was not observed at supraspinal levels. To provide additional evidence for or against separation of mu and delta receptors, an animal model deficient in mu receptors was sought.

The B6CBA^{W-J}/A-Tajp strain of "jimpy" mouse, deficient in brain cerebroside sulfate, possibly required for mu opioid receptor binding, was investigated with B6CBA-A^{W-J} littermates as controls. The jimpy mice were found to be less responsive than controls in tail-flick tests of analgesia to morphine and DAGO, whereas the jimpy and control animals were equally responsive to DPLPE, a delta agonist. Two conclusions were suggested by this study: delta opioid receptors can mediate analgesia in the tail-flick assay in mice, and cerebroside sulfate is not an essential component of the delta opioid receptor.

Another strain of mouse, the CXBK/By strain, is deficient in brain and spinal cord mu opioid receptors. As observed with jimpy mice, the CXBK/By mice showed diminished responses to morphine and DAGO, but full responses to DPLPE in comparison with normal progenitor control strains of mice. In normal mice, there is no cross-tolerance between mu and delta selective agonists in the brain, but in the spinal cord there is cross-tolerance between DAGO (mu) and DPLPE (delta). Significantly, CXBK/By mice respond normally to spinally injected morphine and DPLPE. The data in CXBK/By mice suggest (1) that supraspinal delta opioid receptors can mediate analgesia and (2) there is a difference between brain and spinal opioid receptors.

A third strain of mouse, the C57BL/6Jbg^{J} beige mouse, is hyporesponsive to morphine but responds normally to the delta agonist, DPLPE. The beige mice thus are deficient in mu opioid receptors and were used in experiments to explore molecular mechanisms responsible for the deficient mu receptor-mediated responses. Beige mice were given the cholinergic receptor agonist, carbachol, in their drinking water for 3 weeks. Carbachol treatment improved responsiveness to mu agonists to normal levels. When two groups of mice treated with carbachol for 3 weeks were treated for an additional 3 weeks with either continued carbachol or plain water, both groups lost the responsiveness to mu agonists that the initial treatment with carbachol had provided. These results suggested that beige mice do not suffer from diminished ability to synthesize brain mu receptors, but are not able to maintain functional mu receptors after synthesis. Further experiments revealed that removal of the spleen caused return toward normal mu opioid sensitivity in beige mice. Further, spleen cells removed from beige mice caused decreased mu opioid sensitivity when administered to normal mice.

It therefore appears that the spleen of beige mice may produce a substance, possibly of an immunological nature, capable of reducing mu opioid receptor function in mice. The postulated endogenous factor thus would decrease responses of beige mice to morphine.

Studies by Dr. Vaught and colleagues show that genetically mutant mice can serve as valuable tools in opioid research to separate mu and delta mediated events and to stimulate research into a possible immunological component responsible for diminished responses to mu opioid agonists.

Dr. James Woods, Department of Pharmacology, University of Michigan School of Medicine, Ann Arbor, MI, described an approach to opioid receptors using techniques of behavioral pharmacology in rhesus monkeys. The data obtained in monkeys are generally in agreement with data obtained in other systems. For example, behavioral experiments in monkeys are capable of clearly classifying complex opioid drug effects as morphine-like (including some mixed agonists/antagonists, such as buprenorphine), EKC-like (cyclazocine and certain other mixed agonists/antagonists), or as "pure" antagonists (such as naltrexone).

Opioid receptor antagonists, such as naltrexone, β-FNA, and the more novel mu plus kappa opioid antagonist, quadazocine (WIN 44,441-3), are useful for identification of the types of opioid receptors responsible for, or involved in, opioid drug responses in the monkey. In addition, kappa and mu agonists can be differentiated frequently by their respective patterns of analgesia, discriminative effects, fluid balance, reinforcing properties, development of tolerance, and dependence profiles.

Analgesia is reliably measured by tail-flick and morphine produces dose-related increases in tail withdrawal latency. Kappa opioid receptor agonists, including bremazocine, EKC, MR2033, tifluadom and U-50,488H, are potent and efficacious analgesics in rhesus monkeys. Kappa opioids exhibit stereoselectivity: for example, (+) and (-) tifluadom show different activity. Selective antagonists can tease out different types of opioid receptors. For example, β-FNA does not alter responses to EKC (kappa), but blocks analgesic responses to morphine (mu). Quadazocine, by contrast, can cause dose-related rightward shifts of responses to kappa agonists.

The basic pharmacological concept of multiple opioid receptors and the actions of specific receptor agonists and antagonists are of special importance in assessing the abuse liability of opioids in monkeys. When given an opportunity to self-administer opioids intravenously by pressing a control lever, monkeys readily self-administer many opioids. Many opioids can also suppress signs of abstinence caused by withholding morphine from morphine-dependent monkeys. Careful studies show a direct correlation between potency of different opioids in self-administration tests with their relative ability to suppress abstinence from morphine. Along the same lines, there is also a positive correlation between the subjective discriminative stimulus properties of opioids and potency in self-administration tests. Studies such as these, indicate a common receptor may be involved in analgesia, discriminative stimulus (drug cue) properties, and reinforcement.

As monkeys sometimes terminate self-administration of kappa agonists, drugs acting at the kappa receptor may be somewhat aversive in this species. Monkeys may react to subjective effects of opioids similar to the way humans react.

Tolerance and selective dependence can occur in monkeys with kappa agonists, such as U-50,488H. Even after essentially complete tolerance develops to certain effects of U-50,488H, the sensitivity to morphine with the same endpoints is unchanged, indicating highly selective kappa opioid tolerance.

Abrupt withdrawal of U-50,488H after 3 months of chronic administration leads to a definite abstinence syndrome characterized by hyperactivity, scratching, grooming, yawning, picking at fingers and toes, and other behaviors. The abstinence syndrome is different from that associated with withdrawal from morphine and is not as pronounced. The U-50,488H withdrawal syndrome is precipitated by kappa antagonists, but not by mu antagonists, therefore seems associated with the kappa opioid receptor. The U-50,488H withdrawal syndrome is suppressed by kappa, not by mu, agonists.

These studies in monkeys obviously are of great value in understanding the roles of major types of opioid receptors in behavioral responses to opioids and their possible roles in opioid abuse.

The CPDD symposium on "Multiple Opioid Receptors" brought out several important and interesting features of opioid action. Perhaps the most striking is the ability to identify and classify the major types of opioid receptors, mu, delta and kappa, in an immense variety of preparations ranging from the biochemical level to physiological and behavioral levels. The general agreement concerning receptor identity in a large variety of preparations studied in different laboratories attests to the relative selectivity of the key opioid agonist and antagonist drugs described in this symposium. As the natural opioid ligands are essentially nonselective, it is important that investigators examining opioid receptors use the most selective ligands available.

Evidence for the existence of multiple types of receptors has come primarily from pharmacological studies with receptor selective agonists and antagonists. Other evidence for distinctions between types has come from studies of fundamental regulatory mechanisms, such as effects of sodium and GTP, from species differences, differential anatomical distributions, selective tolerance, genetic differences, from different patterns of dependence and withdrawal, and from different subjective cues in animals. It was evident to all attending the symposium that a great amount of progress has occurred in the past few years of study of opioid receptors. We also anticipate many exciting new discoveries in the years to come.

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FAS: The Need for an Interface

L. Weiner

It is an honor for me to pay tribute to the memory of Dr. Henry L. Rosett, just as it was an honor and privilege to be his friend and colleague for many years. From 1974 until his untimely death in June, 1986, Hank had a sustained and effective commitment to the prevention of alcohol-related birth defects. Hank was a true physician-scientist. As a clinician, he was interested in developing treatment strategies for women who were problem drinkers. As a scientist, he was concerned with gaining an understanding of the mechanisms which underlie alcohol's actions on fetal development. He will long be remembered for the contributions he made.

Hank was a familiar figure at scientific meetings, presenting new findings and evaluating all reports with a view to their clinical relevance. His inquiring mind helped to forge new understandings of the relationships between alcohol and pregnancy. His creativity and ability to synthesize information stimulated new research directions. Hank's integrity and strong sense of caring committed him to focus on the needs of problem drinking pregnant women and their children.

Hank's education and professional experience supplemented his personal qualities. He graduated from Columbia College of Physicians and Surgeons and the New York Psychoanalytic Society. He maintained a private psychiatric and psychoanalytic practice until his death. His research skills were honed on studies of the relationships between cognitive style and career choice. Receipt of the Career Teacher in the Addictions Award provided him with the opportunity to explore the then uncharted field of alcohol and pregnancy.

Hank's greatest contribution may well have been his recognition of the importance of communication and cooperation between researchers, clinicians, and those who make health policy. It is only through an interface that solutions will be found to the complex problems of alcohol abuse in general and problem drinking by pregnant women in particular. But, people working to resolve alcohol problems do not function automatically as an effective network. People operate within the laws of human nature. Each person brings both individual dispositions and training in distinct professional specialities. Scientific progress requires dialogue between these multiple specialities and their unique perspectives with the acknowledgment that each is needed to contribute to successful solutions.

The study of alcohol's effects on pregnancy is no exception to this rule. An historical review of the effects of alcohol on the fetus, which was Hank's first paper in this field, demonstrated the need for interdisciplinary cooperation. The paper traced the scientific community's awareness of the problem of alcohol use during pregnancy and cited conflicts between people with diverse viewpoints over 250 years (Warner and Rosett 1975).

Medical concern about alcohol use during pregnancy began in England when traditional restrictions on distillation were lifted and the country was flooded with cheap gin. In 1726, the Royal College of Physicians and Surgeons petitioned Parliament for control of distilling, calling gin "a cause of weak, feeble and distempered children" (George 1965). Numerous clinical and experimental research reports appeared in both the British and American medical and biological literature throughout the 19th century. By the middle of the 19th century religious and medical temperance efforts had become closely intertwined. Medical writers were influenced by moral attitudes and moral leaders called upon physiologic evidence to strengthen their arguments. Both groups stressed the idea that the sins of intemperate fathers could be visited on their children for several generations.

Reports continued to be published in the U.S. into the beginning of the 20th century. When the 18th amendment was ratified and Prohibition went into effect in 1920, a dramatic drop in medical writing on alcohol and pregnancy occurred. Medical researchers, like many others, believed that alcohol was now a dead issue. With the exception of a few animal experiments, studies on alcohol's effects on offspring virtually disappeared until 1940. When interest in this topic was revived, scientists discounted and ridiculed the pre-Prohibition literature: the early epidemiologic research had "an axe to grind" and the animal experiments were crude and uncontrolled. Although this criticism was valid to a degree, researchers tended to overlook valuable ideas in the medical temperance literature because of the moralistic and unscientific language.

The effects of alcohol on offspring gained renewed scientific attention in the U.S. in 1973 when Jones and associates reported a pattern of malformation in children of chronic alcoholic mothers which they termed fetal alcohol syndrome (FAS) (Jones et al. 1973). Description of the fetal alcohol syndrome was consistent with the many clinical observations made over the past 250 years. The detrimental effects of heavy drinking on fetal development, once widely accepted and subsequently rejected, regained scientific recognition. To avoid repetition of the historical cycle of acceptance and rejection, scientists must work together to define the interacting metabolic, environmental and social variables.

The Medical-Scientific Conference of the National Council on Alcoholism in April, 1975, began the dialogue with the first national workshop on the effects of maternal drinking on child development. Presentations included clinical reports from Seattle and Boston and experimental studies which underscored the complexity of investigating these issues in both animals and humans. To help facilitate the exchange of ideas between the several disciplines conducting research in the area, the Fetal Alcohol Study Group was organized. Members, including practicing physicians, clinical psychologists, and experimentalists trained in basic research, began to meet annually: today's session marks the 13th consecutive year. Hank was responsible for formally initiating this group and was chairman for the first eight years.

The early years saw a re-awakening of scientific interest in the effects of alcohol on pregnancy. There was an enthusiasm about research findings and solutions to methodologic problems that was contagious and all inclusive. Members of the Fetal Alcohol Study Group shared ideas and provided one another with new areas of exploration - with Hank often facilitating the process in late night telephone calls. Investigators with similar interests were linked and new investigators encouraged to join the discussions. Diagnostic criteria for FAS were recommended (Rosett 1980). Skepticism about the existence of FAS lessened as the hardy band of pioneer researchers disseminated their findings.

Hank integrated the literature on the effects of alcohol consumption during pregnancy into a review which served as a background to a 1977 NIAAA conference. This meeting resulted in the first Federal warnings on the adverse effects of alcohol on the fetus. His review was included in the third NIAAA Special Report to Congress on Alcohol and Health (1978).

From this synthesis of the literature, Hank developed a more complex model of the biochemical and pharmacological actions of ethanol than had been considered. He postulated that the fetal alcohol syndrome represents the cumulative results of multiple effects of alcohol consumption on the maternal-placental-fetal system throughout gestation. Alcohol in high concentrations modifies cell functions throughout the body, affecting all organ systems. Effects on fetal development can be both direct and indirect. Biochemical and pathophysiologic effects of ethanol and its metabolites can alter fetal growth and development directly. In vivo and in vitro studies indicate direct effects of both alcohol and acetaldehyde on cell differentiation and growth. Multiple alcohol induced alterations in maternal physiology and in intermediate metabolism of carbohydrates, proteins and fats can alter the environment in which the fetus develops. Retarded growth can be caused by chronic fetal hypoxia, hypoglycemia, or hypothermia, all of which have been experimentally produced by alcohol. Chronic exposure to high doses of alcohol can interfere

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with the passage of amino acids across the placenta and with the incorporation of amino acids into proteins.

Susceptibility of particular organ systems may be greatest at the time of their most rapid cell division. During the first trimester effects of high concentrations of alcohol on cell membrane and cell migrations can disturb embryonic organization of tissue. Throughout pregnancy, disturbances in the metabolism of carbohydrates, lipids, and proteins, and synthesis of RNA and DNA can retard cell growth and division. Large quantities of alcohol can interfere with active transport of amino acids across the placenta reducing availability of essential nutrients. The third trimester is the time of the most rapid brain growth and neurophysiologic organization. High blood alcohol concentrations during this period may impair central nervous system growth and development and limit future intellectual and behavioral capacities. While structural malformations persist, delays in growth and development may be reversible. Some effects will be avoided. This model acknowledged the influence of mediating factors in addition to dose and gestational stage at time of exposure, such as malnutrition, other drug use, stress and parity.

Hank will also be remembered for his pioneering clinical program for heavily drinking pregnant women. Between 1974 and 1979, we conducted a prospective study interviewing more than 1700 women at the time of registration for prenatal care at Boston City Hospital (Rosett et al.,1983b). Women who reported drinking heavily (about 10% of the population) were offered innovative, supportive counseling focused on abstention and integrated with routine prenatal care. Two-thirds of the women who were counseled stopped drinking heavily before the third trimester. Babies born to women who continued to drink were growth retarded and suffered an increased incidence of abnormalities. Benefits were observed in the infants of women who stopped drinking heavily.

The findings at Boston City Hospital suggested the need to motivate primary prenatal care providers to intervene with problem drinking (Weiner et al.,1985). We developed professional educational materials which included basic information on the pharmacology of alcohol and alcohol's effects on fetal development, as well as strategies for obtaining a systematic drinking history and initiating supportive counseling.

Many of Hank's original hypotheses are now routinely accepted and have provided a foundation for later studies. The Fetal Alcohol Education Program at Boston University School of Medicine which Hank and I developed has achieved world-wide recognition and replication. We publish widely, seeking a diverse and broad audience. Alcohol and the Fetus: A Clinical Perspective, published in 1984, provides a unique synthesis of clinical and experimental research with a focus on clinical relevance (Rosett and Weiner 1984). Hank was most proud of the award he received from the Research Society on Alcoholism in 1985 for his overall contribution to the advancement of knowledge about the effects of alcohol on the unborn.

Hank knew that continued progress in research on prevention and treatment of alcohol-related birth defects depends on contributions from and collaboration of many disciplines. We have seen in recent years a proliferation of clinical and experimental reports, leading to a more sophisticated understanding of alcohol's effects. There is still much to be learned in this complex area. We need more knowledge about the mechanisms which cause adverse fetal outcome as well as a better understanding of the pregnant woman who drinks heavily. We must keep our minds open to the multiple factors involved and different strategies proposed. Long term benefits will result from open and objective evaluation.

In this tribute to Henry Rosett, I have focused on his professional contributions. In a short space, it is not possible to fully describe a life as full of accomplishments as his. Nor is it possible to convey what his unique and sensitive personality gave to so many - his colleagues, his patients, his myriad friends and, most especially, his family - his wife, Atholie, and daughters, Amy and Jane.

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Growing Up with Fetal Alcohol Syndrome

M. Russell

I deeply appreciate the honor of being selected as the first to give a lecture in memory of Henry Rosett. As Lynn Weiner (this volume) has so eloquently reminded us, Hank played a major role in establishing the Fetal Alcohol Syndrome (FAS) Study Group and in initiating significant clinical studies on FAS and its prevention. We shall continue to miss him and deeply regret the loss of a productive researcher, a stimulating critic, and a friend.

The title of my talk, "Growing up with FAS," was chosen because it tied together the two main themes that came to mind when I began to think about the current state of the art. One is the degree to which, as the subjects of our prospective studies are growing up, we are able to begin to assess the long-term effects of prenatal alcohol exposure in children as they are developing. Second is the degree to which our field is growing up--that is, our efforts to refine our research questions and methodologies. The adequacy of our measurements and research designs is basic to the state of the art.

Obviously, there is not sufficient time in the next half hour or so to do justice to all the researchers who have contributed to clinical FAS research, let alone to summarize their work. My aim, then, is to discuss in a rather general way the areas of consensus and divergence in the field.

FETAL ALCOHOL EFFECTS IN CHILDREN BORN TO ALCOHOLIC MOTHERS WHO DRINK HEAVILY THROUGHOUT PREGNANCY

For some time it has been well accepted that women in the latter stages of alcoholism who drink heavily during pregnancy give birth to infants who are at risk for fetal alcohol syndrome, defined as having: prenatal and postnatal growth retardation, central nervous system deficits, and distinctive facial features (Rosett, 1980). Many other anomalies, often affecting the cardiovascular, skeletal, and urogenital systems, have also been associated with FAS (Clarren and Smith, 1978; Abel, 1984).

The label 'fetal alcohol syndrome' for the above constellation of birth defects has been challenged on the grounds that it is not unique to alcohol. The fetus can respond to an insult in a limited number of ways. Accordingly, there are other teratogens and conditions, such as phenylhydantoin (Hill, 1976) and phenylketonuria (Lipson *et al.*, 1981), that can produce effects similar to the fetal alcohol syndrome if they are present at critical periods in development. This makes it necessary to rule out these factors and to confirm a history of maternal alcohol abuse during pregnancy to make a definite diagnosis of FAS. At the same time, alcohol is the most commonly used teratogen, and the occurrence of the syndrome can lead to a diagnosis of maternal alcoholism in unsuspected cases (Clarren, 1981). Infants of alcoholic mothers without the full syndrome have an excess incidence of these alcohol-related birth defects and are characterized as having fetal alcohol effects (Rosett and Weiner, 1984). Alcoholic mothers also have a high incidence of spontaneous abortion (Sokol, 1980).

FETAL ALCOHOL EFFECTS IN CHILDREN BORN TO NONALCOHOLIC MOTHERS

The question of how infants of non-alcoholic mothers are affected by drinking during pregnancy is more controversial (Stein and Kline, 1983; Zuckerman and Hingson, 1986). A number of prospective studies of heavy drinking in representative obstetric populations have found an increased incidence of abortions (Harlap and Shiono, 1980; Kline *et al.*, 1980); stillbirths (Kaminski *et al.*, 1981); retarded intrauterine growth (Little, 1977; Little *et al.*, 1986a; Streissguth *et al.*, 1981; Mills *et al.*, 1984); and deficits in cognitive, behavioral, and motor development among infants (Ernhart *et al.*, 1985; Coles *et al.*, 1985; Streissguth *et al.*, 1983a; Richardson *et al.*, 1986; Smith *et al.*, 1986a) and older children (Gusella and Fried, 1984; Streissguth *et al.*, 1980; 1984a; 1984b; Landesman-Dwyer, *et al.*, 1981; Tlucak-Morrow and Ernhart, 1987). However, these findings have not been consistent. Some of the more recent large-scale prospective studies have failed to find a relationship between heavier drinking and such hallmarks of the fetal alcohol syndrome as birthweight (Ernhart *et al.*, 1985; Hingson *et al.*, 1982; Day, *et al.*, 1987). Since consistency is critical to establishing a causal relationship, it will be useful to examine possible sources of these variable findings.

One source of variability undoubtedly stems from difficulties inherent in the measurement of alcohol intake during pregnancy. Since almost all clinical research on drinking during pregnancy is based on self-reported measures of maternal alcohol intake, the reliability and validity of these measures are critical to the interpretation of findings from studies of drinking during pregnancy and adverse effects on the fetus.

A major factor is underestimation. Surveys indicate that self-report data underestimate alcohol consumed in the U.S. by about

40 to 60 percent (Midanik, 1982). There is some evidence to indicate that women report their alcohol consumption more reliably than men (Thompson et al., 1987). If so, this may be because women tend to drink less than men, and infrequent and light drinkers tend to report their alcohol consumption more reliably than heavier drinkers (Armor et al., 1976). On the other hand, women who do drink heavily may be more likely to conceal their high intakes because heavy drinking is less socially acceptable for women than for men.

A second factor influencing the accuracy of self-reports is the time during pregnancy women are questioned. It has been reported consistently that pregnant women tend to deny current alcohol use, such that women asked after the fourth month of pregnancy about their drinking in the first trimester report higher intakes than when asked during the first trimester itself (Little et al., 1977; Robles et al., 1986).

Women interviewed postpartum underestimate their alcohol intakes during pregnancy. Comparison of alcohol intakes reported by women during pregnancy and after delivery in a study at Boston City Hospital found that lower intakes were reported after delivery (Hingson et al., 1982). Factors which are likely to account for the report of higher intakes during pregnancy are better recall, concern for a healthy baby, and greater opportunity for prenatal staff to establish rapport. After delivery the context of the questions about behavior during pregnancy is quite different. They have the aura of "What have you done that might have affected your babies' health," rather than, "How can we help you have a healthier baby." Mothers of infants with problems may consciously or unconsciously minimize their intakes out of guilt about having drunk during pregnancy. Heavy drinkers may fear their babies will be taken from them if they are candid about their alcohol consumption, and memory may be poor or influenced by lowered intakes later in pregnancy.

A final point about the validity of self-reports has to do with the possibility that public health messages on alcohol-related birth defects may deter mothers from admitting heavy drinking during pregnancy. This tendency was documented in a pilot for the Detroit prospective study in which women inadvertently were cautioned about fetal alcohol effects before their alcohol intakes were determined (Nadler et al., 1987). These women reported lower intakes than a comparable group who were questioned about their drinking before hearing about fetal alcohol effects. This clearly demonstrates an effect of intervention on reporting. As ethical researchers it is important to inform study participants of the potential danger of drinking as early in pregnancy as possible. However, real reductions in alcohol consumption reported later in pregnancy, after these warnings, may be confounded by denial. Denial is always a problem, and anything that increases the negative aspects of drinking has the potential to make it worse.

There is a relevant literature on attempts to increase the validity of alcohol measures. Cahalan (1981), the dean of measuring alcohol consumption, advises that people tend to report higher intakes in response to lengthy, open-ended questions that use descriptions of drinking familiar to the respondent. This is in contrast to questions most often found on health inventories, which he characterizes as "nasty, brutish, and short." Accordingly, data from health inventories, such as those used by the Kaiser-Permanente health maintenance organization, may underestimate actual alcohol intakes more than studies focussed on alcohol that have implemented some of the above suggestions. The Kaiser-Permanente data are frequently cited as providing evidence that abortion (Harlap and Shiono, 1980) and lower birthweight (Mills *et al.*, 1984) are associated with having one or two drinks a day, and the above considerations suggest that alcohol consumption may actually be higher than reported. Indeed, only two to three percent of the population reported drinking an average of one or more drinks per day, indicating that these relatively low levels include the heaviest drinkers in the population (Mills *et al.*, 1984).

It is a truism that the more questions you ask about alcohol intake, the higher the intake--the amount goes up for every extra "yes." At least two current approaches to measurement take advantage of this principle. Day's study in Pittsburgh gets quantity-frequency data, not only on the usual amount drunk, but also for amounts more than usual and less than usual. Studies of Sokol, *et al.* (1985) in Cleveland and Detroit utilize a 14-day recall. Although I have colleagues who argue this point, some studies assume that since alcohol consumption tends to be underestimated, the higher the quantity reported, the more likely it is to be valid (Midanik, 1982). To the extent that this is so, it seems reasonable to suppose that these approaches increase the validity of reported consumption.

Lowe (1986) recommends using a "bogus pipeline" approach to improve validity. In his study from Birmingham, Alabama, pregnant women who thought their self-reports of alcohol consumption would be checked by an objective, physiologic test, reported drinking almost twice as often as those who didn't, 27% compared to 14%. This technique has been implemented in the Pittsburgh prospective study.

Ruth Little *et al.*,(1986) have examined the usefulness of laboratory tests in measuring alcohol and other substance use in women postpartum. They confirm the prevailing opinion that currently available laboratory tests for alcohol consumption are not sufficiently specific or sensitive to replace self-report data, although they may be useful in supplementing it. For example, a positive laboratory finding might help break down denial in some women. However, failure to confirm self-report of heavy drinking with laboratory tests is more likely to reflect the lack of sensitivity of the laboratory test, rather than overestimation of her drinking by the respondent.

Pregnancy itself affects drinking. The effect of pregnancy on the validity of reported alcohol consumption is confounded by its effects on the actual intake. There is a spontaneous tendency for women to reduce their alcohol intake when they become pregnant (Hook, 1976; Little et al., 1976), and reductions are also made consciously, out of concern for a healthy baby. These tendencies are influenced by mothers' original drinking levels and by whether or not she is alcoholic (Little and Streissguth, 1978).

Reductions in drinking during pregnancy tend to be proportional (Little et al., 1976). That is to say, women who drank heavily prior to pregnancy are likely to continue drinking more during pregnancy than women who drank infrequently or lightly prior to pregnancy, even though both may reduce their alcohol consumptions. Alcoholic women also tend to reduce their intakes during pregnancy, but they may increase binge drinking, (Little and Streissguth, 1978), and studies in animal models suggest that binge drinking during critical periods of development can be harmful to the fetus (Sulik et al., 1981).

What about validity? Can we believe women who say they reduced their drinking during pregnancy? The tendency for women to reduce their alcohol intake during pregnancy was well documented before there was any widespread report of alcohol-related birth defects. Therefore, it is probably safe to assume that in the majority of cases, the reductions reported are real. Women who are highly motivated to continue drinking, pre-alcoholic or alcoholic drinkers, who are most likely to continue drinking heavily or binge drinking, may be unwilling to report this behavior to health professionals who have indicated that heavy drinking is dangerous to their babies. However, reductions have been validated by collateral reports in the Atlanta prospective studies, and others have noted improvements in maternal appearances, weight gains, and blood chemistries associated with reported reductions.

Although denial tends to reduce the sensitivity of self-reports in identifying women who drink heavily during pregnancy, self-report tends to be highly specific. There is little reason for pregnant women to exaggerate their alcohol intakes, and it seems reasonable to believe that women who report heavy drinking drank at least as much as they say they did. Even so, for all but intakes that approach lethal limits, there is room for underestimation.

How does the issue of validity influence our interpretation of the clinical research on FAS? It suggests that one must keep in mind, not only the amount of alcohol reported, but also the following: time during pregnancy of the alcohol intake, time during pregnancy the alcohol intake is reported, whether the woman is alcoholic or has indications of problem drinking, whether the woman has been sensitized to the relevancy of her drinking regarding possible harm to the fetus, and whether intake data are gathered in a way that promotes accurate reporting. Variability in the available studies regarding these factors could account for some of the lack of consistency in their findings.

The timing of measurements as they relate to critical periods during pregnancy may also contribute to variability in study findings. This is an issue of great importance since it determines the efficacy of intervention at various points during pregnancy. The first trimester is the period of organogenesis, during which alcohol exposure produces an increased incidence of congenital malformations, distinctive facial features, hearing abnormalities, and abnormalities in early CNS development (Rosett and Weiner, 1984). Growth in body size takes place, for the most part, during the second and third trimesters, and drinking during these periods would influence birth weight and length. The brain is smooth at four months gestation, and rapid growth during the latter half of pregnancy results in the development of normal corrugations in the surface and is characterized by active dendritic branching, synapse formation, and myelination--all critical to later function.

The differentiation of fetal alcohol effects by period of exposure is a very active field of research using animal models. Clinical studies that have measured alcohol intakes in every trimester have found, however, that in human populations, much of the variability in drinking during pregnancy is accounted for by drinking prior to pregnancy, or embryonic drinking. Thus, some researchers have found drinking prior to pregnancy relates more strongly to adverse pregnancy outcomes than drinking during pregnancy itself (Hanson et al., 1978; Little, 1977; Wright et al., 1983).

A major factor in accounting for these findings is the fact that drinking patterns prior to pregnancy generally persist up until pregnancy is recognized, from six to eight weeks after conception, overlapping with much of the period during which embryonic development takes place (Moron et al., 1985). In heavier drinkers whose alcohol intake interferes with regular menstrual periods, pregnancy recognition may be delayed even more, prolonging this early period of exposure (Russell and Czarnecki, 1985). Women usually come in for prenatal care toward the end of the first trimester. Their drinking at this time tends to be more like drinking during the second trimester. This factor, together with women's reluctance to report current drinking during pregnancy, may also contribute to failure in some studies to observe associations between first trimester drinking and poor pregnancy outcome.

In Atlanta, Coles et al., (1985) have been able to match women who continued drinking throughout pregnancy with a comparable group which stopped drinking in the second trimester. Compared to unexposed infants, infants exposed to alcohol at any time during pregnancy had alterations in reflexive behavior, less mature motor behavior, and increased activity level, as measured by the Brazelton Neonatal Behavior Scale. In addition to effects seen in infants exposed through the second trimester, infants exposed throughout pregnancy were inferior in observed state control, need for stimulation, motor tone, tremulousness, and asymmetries in reflexive behavior.

This is consistent with what might be expected based on the animal research; however, alcohol exposure during the third trimester is associated with other factors that may increase the vulnerability of the fetus to alcohol effects. Smith *et al.*, (1986) found in the Atlanta population studied above that continuing to drink throughout pregnancy was also associated with a history of alcohol withdrawal and/or abstinence syndrome, length of drinking history, reported tolerance to alcohol, drinking by siblings, and drinking most often with other family members. This suggests that drinking during the third trimester may be symptomatic of prealcoholic problem drinking or alcoholism.

Sokol *et al.*, (1981) included the Michigan Alcohol Screening Test, more popularly known as the MAST, in their Cleveland prospective study. As expected, they found that fetal alcohol syndrome was most strongly associated with persistent exposure of the fetus throughout pregnancy (Sokol *et al.*, 1986). In addition, positive MAST scores also accounted for a significant portion of the variability in Fetal Alcohol Syndrome. In our Buffalo data, we measured alcohol intake prior to pregnancy using a self-administered questionnaire and found an increased risk of spontaneous abortion associated with heavy drinking, but no effect on intrauterine growth or Apgar scores in the newborn, (Russell, 1985) or physical, cognitive, or behavioral development at age six years (Russell *et al.*, 1987). By contrast, we do see deficits in head circumference at birth and in cognitive development at six that are related to maternal indications of problem drinking.

These observations are consistent with European studies by Majewski (Majewski, 1981) and Olegard (Olegard *et al.*, 1979) that indicate that the effect on the fetus of a given alcohol consumption is greater if a woman is in the chronic stage of alcoholism than if she is in the prodromal state. They have hypothesized that women in the chronic stage of alcoholism may pattern their drinking to maximize blood alcohol levels and enhance its intoxicating effect, for example, by not eating when they drink. Also, women in the latter stages of alcoholism may metabolize alcohol less well, which could produce higher blood alcohol levels and extend the period of exposure to these higher levels. If acetaldehyde metabolism were also affected, this could produce greater fetal injury since acetaldehyde is much more toxic than alcohol.

It is not clear from these studies whether third trimester effects in humans are related solely to alcohol exposure during this critical period of development or if maternal alcoholism increases fetal vulnerability. To the extent that only alcoholic or prealcoholic women continue to drink throughout pregnancy, it may be difficult, if not impossible, to disaggregate these effects in humans. Even in studies of alcoholic women who do and do not drink throughout pregnancy, there could be unmeasured differences that determine whether or not they continue to drink and also affect fetal well-being.

OTHER FACTORS INFLUENCING THE EFFECT ON THE FETUS OF A GIVEN ALCOHOL EXPOSURE

Maternal alcoholism is not the only factor that may influence fetal susceptibility to alcohol exposure. Differential vulnerability is a very "hot" area of research at the moment, and it may account for some of the lack of consistency in our clinical studies and help us understand why only some children of heavy drinking alcoholics are affected.

Sokol et al., (1986) hypothesize that blacks are more vulnerable to alcohol-related birth defects than whites and have published data showing that infants born to heavy drinking black women are more likely to be small for gestational age and seven times more likely to have the full fetal alcohol syndrome than similarly exposed infants born to whites. In their Cleveland data, they have also found a lower threshold for alcohol-related anatomic abnormalities among blacks, for whom incidence increases above four or more drinks per day, compared to a threshold of six or more drinks per day for whites (Sokol et al., 1987). Results for craniofacial and other anomalies were similar. This may have implications for alcohol exposure thresholds as they relate to child development as well, since Ernhart et al., (1987) found a relationship between facial anomalies associated with embryonic alcohol exposure and deficits in cognitive, psychomotor, and language development up through age three years.

Abel's (1984) reviews of FAS case reports found that women giving birth to FAS babies tended to be older and of higher parity. He designed animal experiments to differentiate between these two possibilities, and concluded that pregnancies among older mothers were at higher risk for alcohol-related birth defects (Abel and Dintcheff, 1984; Abel and Dintcheff, 1985). More recently, a study of alcohol use in primiparous women over age 30 reported significantly lower scores on the Bayley Mental Development Index and more physical anomalies associated with heavy drinking prior to pregnancy (O'Connor et al., 1986).

Genetic differences in vulnerability to a given alcohol dose have been demonstrated in inbred strains of mice and rats (Chernoff, 1980; Gilliam et al., 1987). Differences between dizygotic twins in the fetal effects of alcohol exposure suggest that there are also genetic differences in vulnerability in humans (Christoffel and Salafsky, 1975). There have been few, if any, clinical studies to investigate genetic factors in FAS. However, Brien et al., (1983) have demonstrated substantial individual differences in the disposition of alcohol among six healthy women, 16 to 18 weeks pregnant. These differences could play a major role in determining the level and length of fetal exposure to alcohol and acetaldehyde, and there is evidence to suggest that the disposition of alcohol is at least partly under genetic control.

There is also renewed interest in the role of paternal drinking and how it may relate to some of the outcomes that are being examined with respect to maternal drinking during pregnancy. It is unusual for a woman to drink more than her mate. Therefore, fathers of children born to women who drink heavily during pregnancy are also likely to drink heavily. Little and Sing (1986) recently reported an association between fathers' drinking and lowered birthweight; however, a second group was unable to replicate this finding. Several investigators have found a negative correlation between children's IQs and their fathers' drinking (Gabrielli and Mednick, 1983; Werner, 1986; Ervin et al., 1984). Although recent animal studies suggests that this influence may be mediated via a direct effect on the sperm (Lee et al., 1987; Moore et al., 1987; Tan, et al., 1987), it is generally thought to be a genetic effect. In either case, it represents an additional factor to be taken into consideration when evaluating the effect of maternal drinking on child development.

Clearly, there may be other factors that may affect fetal vulnerability to alcohol exposure, but I want to save some time to mention a few significant studies of fetal alcohol exposure and child development that I have not already discussed.

Spohr and Steinhausen (1987) have extended their follow-up of FAS children. Their subjects now have a mean age of eight years. They have a fairly large sample of 54 children, including some less severely affected cases, which they feel complements earlier work (Darby et al., 1981). They found a general trend of improvement in physical symptoms and psychiatric findings. IQs stayed about the same for those below 70 or above 115, but tended to increase between 70 and 115. However, the children still had higher abnormality scores and a number of relevant psychiatric symptoms showing no significant change, especially hyperactivity. Persisting handicaps were most obvious in looking at educational status; only six children, 17% of those school age, attended "normal" school, and two of these attended normal schools specializing in hearing defects and speech disorders. 51% attended schools for the educationally subnormal, and 20% attended a training center only. At least 4% were severely subnormal and without any education. Severity of the morphologic damage at the initial exam correlated with educational status, with less severely affected children tending to be in the normal schools.

There are fewer follow-up studies in children of nonalcoholic mothers; however, the Seattle Longitudinal Study is unique in having the longest follow-up of a prospectively identified group of children. Ann Streissguth et al.,(1986) reported that, between seven and eight years of age, early prenatal alcohol exposure was most significantly related to continuous performance test errors of commission, reaction time, and the vigilance errors summary score. These results are consistent with earlier reports of alcohol-related attention deficits in this population at one day of life and at four years, and they are also consistent with findings from animal models. By way of contrast, Morrow-Tlucak and Ernhart (1987) found that maternal alcohol and marijuana use during pregnancy was associated with reductions in activity, emotional reactivity, irritability and dependence, and increased rigidity and unusual task absorption--counter to what would be expected. Clearly, more studies are needed! No state-of-the-art lecture would be complete without that phrase!

This has been a brief overview of some of the measurement issues and research questions that influence the current state of the art in clinical FAS research. It suggests that we should not be lulled into a possibly false sense of complacency by surveys which find low levels of drinking among pregnant women. While the evidence indicates that women are reducing their alcohol intakes early in pregnancy (Smith et al., 1986c; Fried et al., 1984; Streissguth et al., 1983b), it is not clear that this is true for women at highest risk. Women in the chronic stages of alcoholism who have lost control over their drinking and are dependent on alcohol may be less likely to spontaneously reduce their alcohol intakes to "safe" levels during pregnancy and less responsive to intervention efforts. Even intelligent, well-educated women, who are very health conscious during pregnancy, may drink heavily prior to pregnancy, and current data suggest that this may represent a risk to fetal well-being.

The methodological issues discussed suggest that control groups of abstinent and light, infrequent drinkers may be contaminated by heavy drinkers who underreport their intakes. This has the potential to make it more difficult to detect real differences related to alcohol consumption and may make tests of significance more conservative. Balanced against this is the tendency for levels of alcohol consumption in the exposed group to be underestimated. This leads the unwary to conclude that alcohol-related birth defects are associated with lower levels of alcohol intake than, in fact, they are. Alcohol classifications that lump all the heaviest drinkers into a single group with a rather low cut point may also suggest to the naive reader that defects are associated with lower alcohol exposures than are typical of the affected cases.

In designing studies of alcohol-related birth defects, one must strike a balance between what is perfect and what is possible. It is impossible to conduct the "perfect study" of alcohol-related birth defects in humans. Such a study would require the accurate measurement of all the factors that could potentially influence pregnancy outcome. Any such effort would not only be prohibitively expensive, but also so intrusive that it could not fail to modify some of the behaviors/exposures under study. Finally, if potentially harmful behaviors were not spontaneously modified, there would be an ethical imperative to intervene in all but the most benign circumstances. Indeed, we are already intervening to the best of our ability by cautioning the public regarding the dangers of drinking during pregnancy.

My intention, in mentioning these limitations, is not to end this state-of-the-art lecture on a negative note, but to put it into perspective. Findings from studies of alcohol-related birth defects in nonalcoholic populations are inconsistent and must be interpreted cautiously. However, fetal alcohol effects have been reported from a number of carefully conducted studies, and the public health significance of these findings is such that it is very important to continue our efforts to achieve a better understanding of the factors that influence them. FAS researchers have done much to refine their measures, their analyses, and their research questions, and these efforts are continuing.

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The Dilemma of Cocaine Exposure in the Perinatal Period

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Cocaine's pharmacological action including decrease in uterine blood flow, increase in uterine vascular resistance, and reduction in fetal oxygen levels contraindicate its use in pregnancy. The vasoconstriction, tachycardia, and increased blood pressure found to be associated with perinatal cocaine use increase the chance for intermittent intrauterine hypoxia, preterm labor, precipitous labor, and abruptio placenta. In spite of some animal data and limited human studies, more research is essential to further evaluate cocaine's effect upon the perinatal period, the infant and the child. The following summarizes these issues: 1) Maternal medical and obstetrical complications need further delineation with reference to nutrition, disease entities and appropriate treatment regimens. 2) There is a need to develop epidemiological studies in order to determne if cocaine exposure in-utero influences formation of congenital malformations and the incidence of Sudden Infant Death Syndrome. 3) By clinical and ultrasound measures, the effects of cocaine upon brain growth and brain pathology must be evaluated. 4) The neurobehavioral outcome of neonates exposed to cocaine in-utero via clinical measurement scales such as abstinence scoring and the Brazelton Neurobehavioral Scale need more precise definition. 5) The potential long-term developmental effects upon the infant and child must be further delineated. 6) Psychosocial situations with regard to parenting abilities and child protection in cocaine abusing families must be evaluated.

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Fetal Alcohol Effects: Central Nervous System Differentiation and Development

J. West

There is evidence indicating that in spite of considerable publicity regarding the risk to the fetus from heavy maternal drinking (Surgeon General, 1981; Rosett and Weiner, 1984; Little et al., 1984), alcohol-induced birth defects (ARBD) remain a serious problem. Mental retardation is the most devastating feature of the fetal alcohol syndrome (FAS), and it is now known that it can occur in the absence of other FAS features (Majewski et al., 1976). A recent paper by Abel and Sokol (1987) indicates that maternal alcoholism is now the leading known cause of environmentally-induced mental retardation in the Western world, surpassing even Down's syndrome and cerebral palsy as a cause of CNS-related birth defects. Therefore, the study of CNS fetal alcohol effects remains an important area on which to focus research. This paper will review some of the relevant neuromorphological findings demonstrating the effects of alcohol exposure on the developing brain.

The human literature focused on structural damage to the brain resulting from prenatal exposure to alcohol is sketchy. Only a few brains from fetuses and infants diagnosed as exhibiting FAS have been examined (Clarren et al., 1978; Clarren, 1981, 1986; Majewski et al., 1978; Peiffer et al., 1979; Wisniewski et al., 1983). Fetal brain tissue is often damaged at delivery so that it is inadequate for neuropathological evaluation of fetal alcohol effects (Clarren, 1986). Furthermore, because death in these children was often due to severe cardiac problems, there is a concern that these brains represent a skewed sample. Nevertheless, some interesting findings have been reported and they may give us some clues as to the type and location of deficits in FAS animal studies.

Many of the human FAS brains were smaller and some of them had neuroglial heterotopias with viable ectopic cell clusters (Clarren, 1986), suggesting that alcohol may not only be neurally toxic, but may also specifically interrupt the migration of neurons after they are generated. Other brains exhibited enlarged ventricles and small or nonexistent commissures, indicating either underdeveloped commissural systems or white matter lesions (Clarren, 1986). The exact amount of alcohol required to produce such damage and the timing of the insult for causing specific defects are questions that remain unanswered.

Since many women who consume large amounts of alcohol during pregnancy also abuse other drugs, may suffer from nutritional deficits, and may not accurately report how much alcohol they had consumed during pregnancy, animal studies have been used to answer important questions associated with CNS-related fetal alcohol effects. Although data derived from some of the human FAS autopsy material suggesting that alcohol could affect the generation and migration of neurons have been available for nearly a decade, it has been only during the past year that experimental evidence has been forthcoming to support such a hypothesis. Miller (1986, 1987) found that the "birth" of corticospinal neurons was delayed and that they sometimes migrated to the wrong cell layer. Interestingly, there also were more of the cells, suggesting that the normal process of paring down the number of neurons (normally occurring cell death) was impaired.

Parenthetically, this latter conclusion is consistent with a report of a significant lag in the rate at which non-myelinated axons of the optic nerve of animals exposed to alcohol during gestation were lost in association with the overproduction of neurons (Samorajski, Landcaster and Wiggins, 1986). In most brain regions during development there is a normal overproduction of neurons followed by a loss of those cells that do not make appropriate contacts, which appears to be a mechanism to permit an optimal number of neurons while avoiding errors in connectivity. This ability may be impaired as a consequence of fetal alcohol exposure.

It has been hypothesized that alcohol's main effect on development is primarily a function of interfering with cell division (Pennington *et al.*, 1983). However, the limited cell count data available do not corroborate that idea very well. Cell count studies in the hippocampus have revealed alcohol-induced deficits in field CA1 following exposure to alcohol during days 10-21 of gestation (Barnes and Walker, 1981), and in field CA4 following early postnatal exposure (West *et al.*, 1986). Interestingly, in both cases the population of granule cells in the dentate gyrus, which undergoes most of its neurogenesis during the first three weeks of postnatal life (Bayer, 1980), was either unaffected or *increased*. In the cerebellum, it also appears that the population of large Purkinje cells that were generated prior to early postnatal alcohol exposure was more vulnerable to alcohol than the population of granule cells which was in the process of division at the time of the alcohol insult (Phillips and Cragg, 1982).

One of the most common effects of alcohol exposure during development appears to be a general delay in growth and maturation. Restrictions in overall brain growth are quite common in response to alcohol in both humans and animals (Samson, 1986). One of the clinical signs of FAS is microcephaly (a small head for body size); its corollary, microencephaly (a small brain for body size), is an indicator of mental retardation. Microencephaly also has often been reported as a fetal alcohol effect (FAE) in animals, although there is evidence that it may be associated only with alcohol exposure during the third trimester unless accompanied by undernutrition (Samson, 1986).

Not surprisingly, neurons in these developing brains tend to be smaller and have poorly developed processes. Pyramidal cells in the cortex (Hammer, 1986) and hippocampus (Davies and Smith, 1981) exhibit smaller somas and stunted dendritic growth with fewer dendritic branches and spines. Importantly, evidence of delayed neuronal development other than simply stunted growth has been reported. Volk (1984) demonstrated that perisomatic processes, which normally protrude abundantly from the somas of cerebellar Purkinje cells in eight-day-old rats, but are absent by postnatal day 12, are still present at that time in rats that were exposed pre- and postnatally to alcohol.

Alcohol exposure during development not only produces neurons with stunted dendritic arbors and fewer dendritic spines, but some of the surviving spines are aberrantly shaped (Hammer, 1986). Dendritic spines are perhaps the most dynamic and variable element of dendritic structure. Spines were originally thought to be a simple mechanism for increasing dendritic surface area. More recently, it has been shown that spines are much more involved functionally and that they can provide a means for adjusting synaptic potency (Rall, 1978). Long thin spines have larger stem resistance values matched to higher input resistances. Longer, thinner spines can cause greater attenuation of incoming signals (Wilson, 1984). Therefore, in addition to simple measures such as the number of spines present, the diameter and length of the spine shaft are key morphological factors in determining the consequences of alcohol exposure on developing neurons (Hammer, 1986).

Few studies have investigated the effects of alcohol on neuronal development at the ultrastructural level. However, the cytoplasm of Purkinje cells in 12-day-old rats exhibits fragmented rough endoplasmic reticulum with large quantities of free ribosomes (Spohr and Stoltenburg-Didinger, 1985). Furthermore, photomicrographs of the cerebellar molecular layer, stained with ethanolic-phosphotungstic acid, showed a reduced number of stained synapses (Volk, 1984). Other reports, however, indicate that synapse formation may be delayed less by alcohol than other developmental processes (Hoff et al., 1984; Jones and Colangelo, 1985). Considering the paucity of data available, it is not known whether the reported difference is a function of regional vulnerability, regimen of alcohol treatment or the ages of the animals when examined.

It is essential to realize that when conducting studies directed toward detecting the effects of alcohol on the developing brain, there are key points that must be kept in mind. While it is important to address the questions of when alcohol affects the brain during development, it is not appropriate simply to divide the gestation period of the species being examined into three equal periods and expose the fetus to alcohol during one or more of these three so-called "trimesters." In order to extrapolate the results from animal studies to humans, it is necessary to consider equivalent periods of brain development of the two species. Although all mammals pass through the same stages of brain development, the timing of those stages, relative to birth, can be quite different (Dobbing, 1981; Dobbing and Sands, 1973, 1979). When the timing of the alcohol exposure is considered, different effects may occur as a consequence of alcohol exposure during different "trimesters equivalent" (West, 1987). Furthermore, there is evidence that the third trimester equivalent may be a period when the brain is especially vulnerable to alcohol (Phillips and Cragg, 1982; West and Hamre, 1985).

Another key question is whether there are regional differences in susceptibility of the brain to the deleterious effects of alcohol exposure. Although there is surprisingly little information available on the subject, the data that are available indicate that various regions of the brain are affected differentially by alcohol exposure (Sulik et al., 1984; Pierce and West, 1987).

In spite of the already extensive fetal alcohol literature, we still have many questions concerning the permanency of fetal alcohol effects. Most of the FAS patients that were identified in the 1970s are just now teenagers. Although it appears that the mental retardation in these patients is persisting (Streissguth et al., 1985), we know almost nothing about the long term consequences of exposure to alcohol during development.

The morphological data derived from adult animals exposed to alcohol during different stages of development are meager and they give a conflicting message. Abel and his associates (1983) found a decrease in the number of dendritic spines and a shift in the type of spines on pyramidal cells in field CA1 of the hippocampus of adult rats treated with alcohol in utero. On the other hand, Pentney et al. (1984) did not find alterations in dendritic branching of cerebellar Purkinje cells. That finding is quite remarkable considering the number of studies that have found extensive damage to the cerebellum in younger animals that were exposed to alcohol during development (Bauer-Moffett and Altman, 1977; Phillips and Cragg, 1982; Volk, 1984; Pierce and West, 1987).

A thorough understanding of the relationship between the structural and functional changes associated with fetal alcohol exposure will provide the foundation for understanding the mechanisms that produce fetal brain damage, and ultimately for providing treatment regimens for preventing or reversing fetal alcohol effects. Such research begs our attention.

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Genetics and the Specific Dimensions of Risk for Alcoholism

M. Schuckit

INTRODUCTION

Alcoholism is a genetically influenced disorder. Detailed reviews published on almost an annual basis have documented the familial pattern of this problem, the probable increased concordance among identical twins, and the four-fold increased risk for alcoholism in children of alcoholics, even when they were adopted out at birth and raised without knowledge of their biological parents' problems (Goodwin 1985; Schuckit 1985a; Schuckit 1986a). Despite this evidence, it is important to remind ourselves that the genetic factors are likely to be complex, and that there is at least one twin and one adoption study that do not support the other results (Roe and Burks 1945; Murray et al., 1983). It is also essential to recognize that genetic influences only explain part of the risk for alcoholism, and that environmental events must also play a role (Peele 1986; Cadoret et al., 1987; Beardslee et al., 1986).

The preponderance of positive results in genetic studies has spawned further work attempting to identify what might be inherited to increase the alcoholism risk. Most of these investigations have studied young individuals with enhanced vulnerability for alcoholism, characterizing them before alcohol-related life problems and associated treatments obscured any pre-existing clinical picture. Like the genetic studies themselves, these approaches have also been reviewed in depth in recent years, and their liabilities as well as their assets must be kept in mind (Schuckit 1985b; Schuckit 1985c). The high potential cost and the probability that only 1 out of 3 sons of alcoholics will actually develop alcoholism are balanced by the ready availability of such populations for study and the hope that these efforts might eventually improve prevention and treatment approaches.

The present manuscript is not a simple repetition of the recent reviews, but places an emphasis on a limited number of topics relevant to both studies of populations at high risk for alcoholism and a discussion of psychiatric aspects of alcohol and drug abuse. The following sections review some aspects of the relationship between alcoholism and other psychiatric illnesses and the possible importance of alcoholic subtypes, present recent developments emanating from protocols evaluating individuals at high risk for alcoholism, and outline a number of issues for the future.

SOME CONCEPTUAL ISSUES

Establishing accurate diagnoses in most areas of health care requires the use of both historical data and relevant laboratory tests to indicate a probable prognosis and optimal treatment (Goodwin and Guze 1984). Psychiatry, unfortunately, has few (if any) relevant biological diagnostic tests, and thus places almost total emphasis on the past and present clinical pictures. However, our field is handicapped by the observation that similar behavior is often seen in the context of multiple disorders. Thus, severe and relatively persistent psychotic symptoms resembling schizophrenia are likely to be seen in the context of ongoing abuse of stimulants, and anxiety syndromes resembling panic disorder or generalized anxiety disorder can frequently be observed during withdrawal from brain depressants (Schuckit 1984a; Schuckit 1983b). Such "dual diagnosis" patients raise the question of whether two or more independent disorders are present, or if one picture represents a symptom cluster inherent in the other disorder. In the latter case, it is logical that successful treatment of the primary problem could result in the disappearance of the second symptom pattern. This is a key issue in genetic research because the inclusion of multiple hereditary problems in the same clinical sample can make it difficult, if not impossible, to isolate any genetic influences for either disorder. The philosophy used in our laboratory has been to limit study to the individuals with the more straight-forward diagnoses, e.g., the 70% or so of alcoholics who have no obvious pre-existing psychiatric disorder and thus can be said to have primary alcoholism (Schuckit 1984a; Schuckit 1986b). For at least two psychiatric disorders this becomes a potentially important consideration in the study of the genetics of alcoholism.

The relationship between affective disorders and alcoholism is too complex to generate a sure answer or to produce a general rule that is correct 100% of the time. However, it can be clinically and scientifically useful to establish a heirachy where, until proven otherwise, it is assumed that even major depressive episodes occurring only in the context of substance abuse should be considered secondary and are likely to disappear with time alone (Schuckit 1986b). Similarly, patients with major depressions antedating the severe alcohol problems should be considered to have primary affective disorder, not primary alcoholism. As discussed in a recent review, this is relevant because approximately 90% of alcoholics in treatment give a history of severe sadness at some time in the past (a rate perhaps no higher than other patients coming to treatment with major life problems); one in three alcoholic men have experienced a severe and persistent depression fulfilling criteria for major depressive disorders at some time in the past (but most of these were temporary and only occurred in the context of heavy drinking); but in all likelihood only about 5 percent of alcoholic men and perhaps 15 percent of alcoholic women demonstrate major depressive episodes independent of their heavy drinking (Schuckit 1986b; Schuckit and Winokur 1972; Powell et al., 1987). Thus, it is probable that the rate of independent major depressive disorders is no higher among alcoholics than the general population. Family studies are also consistent with the probability that in most families alcoholism and bipolar affective disorders are not related (Dunner et al., 1979), and evaluations of adult children of alcoholics adopted out close to birth have failed to document an increased rate of major depressive disorder or mania (Schuckit et al., 1972; Goodwin et al., 1974; Cloninger et al., 1984; Cadoret 1980). Also supporting the probable distinction between alcoholism and depressive disorder are follow-up studies of alcoholics that demonstrate that those who have been diagnosed as having primary affective disorder with secondary alcoholism appear to run a course that is different from primary alcoholics (Schuckit and Winokur 1972; Schuckit 1986b; Powell et al., 1987), and the finding that the major predictor of depressive symptoms in alcoholics, other than an independent family history of depression, is the quantity and frequency of drinking (Brown 1987). Thus, Angst (1966) and Cloninger et al., (1983) each concluded that alcohol and depressive disorder appear to congregate in different families and show little evidence of genetic linkage. As a result, it is important in genetic studies to focus only on primary alcoholics and to consider separately men and women with primary affective disorder antedating the secondary alcoholism.

A second conceptually important diagnosis is the antisocial personality disorder (ASPD). A potentially confusing clinical picture occurs when an alcoholic demonstrates periods of violence and criminal behavior, raising the possibility of the presence of an ASPD. Contributing to the dilemma is the observation that perhaps 70 percent or more of men fulfilling criteria for ASPD develop severe enough alcohol-related problems to be labelled as secondary alcoholic during their teenage and early adulthood years (Schuckit 1973; Vaillant 1982; Vaillant 1984). Carefully defined ASPD also appears to be genetically influenced (Crowe 1972; Cloninger et al., 1975; Cloninger et al., 1983; Cloninger and Reich 1981). However, while antisocial problems can be seen among practicing alcoholics and while ASPD patients are likely to have secondary alcoholism, there are a number of indications that primary ASPD and primary alcoholism are two independent disorders. First, there is little evidence of increased rates of primary alcoholism in the families of men with bonafide ASPD (Hesselbrock et al., 1985; Vaillant 1982). Second, the adoption studies of children of alcoholics do not demonstrate an increased risk for ASPD (Cloninger et al., 1983; Goodwin et al., 1974; Bohman 1978), and there is evidence that children of ASPD subjects who are adopted out demonstrate high rates of ASPD, not primary alcoholism (Cadoret et al., 1985; Cadoret et al., 1987). Finally, on follow-up, individuals with primary ASPD and secondary alcoholism appear to have a clinical course distinct from primary alcoholics (Schuckit 1985d; Hesselbrock et al., 1986; Vaillant 1982). Thus, Cloninger and colleagues (1981) as well as others, have concluded that family and adoption data indicate a surprising degree of independence between genetic factors that predispose to alcoholism and those that predispose to ASPD.

Similar conclusions can probably be drawn regarding the independence of primary alcoholism and other genetically influenced disorders, including several of the major anxiety diagnoses outlined in DSM III (American Psychiatric Association 1980; Schuckit 1984a). The point of this methodological review is not to prove that alcoholism, ASPD, and depressive disorders are definitely separate illnesses. The emphasis is on the possibility that in most families they are independent genetic disorders, although symptoms observed in one illness are also likely to be seen in the others. Because of this possibility and as a reflection of the rather primitive tools available for studying genetic factors in alcoholism, it makes sense to limit most genetic investigations to relatives of alcoholics who developed their alcohol-related symptom complex before the onset of any other major psychiatric disorder: (i.e., primary alcoholics).

An additional factor that needs to be considered in developing studies of populations at high risk is that there are probably subtypes among alcoholics with different patterns of interactions between genetic and environmental influences. One such possible subgrouping has been presented by Cloninger and colleagues (Cloninger et al., 1984; Cloninger 1987). They outline an early onset male predominant type with high levels of genetic influences, a severe clinical course and relatively weak sensitivity to environment, as well as a second type with a complimentary pattern of characteristics. It is also possible that there are other subtypes differentiated by gender, patterns of brain waves, responses to alcohol, and so on (Pollock 1986). More data will be required however, before these potential groupings can be optimally used.

The data presented in the next sections must be considered in light of all of the methodological issues discussed above. Indeed, considering the difficulties inherent in using a history to accurately diagnose the alcoholic parent, the possible heterogeneity within samples regarding the presence of multiple psychiatric disorders within the families, and the almost certain existence of alcoholic subtypes, it is impressive that the studies of populations at high risk for alcoholism have produced such consistent results.

AN UPDATE OF STUDIES OF POPULATIONS AT HIGH ALCOHOLISM RISK

Reviews of investigations of children of alcoholics published up to 1986 have highlighted several potentially important findings associated with a family history of alcoholism. The following comments are organized around the major findings; first, giving a summary of prior results, then expanding with information presented over the last two years.

Our laboratory first documented a decreased intensity of reaction to three to five drinks of ethanol in sons of alcoholics in 1976, a finding which was corroborated by additional studies in the United States and Scandinavia (Schuckit 1985a; O'Malley and Maisto 1985; Mednick 1982). This decreased intensity of subjective feelings after drinking has recently been replicated among sons of alcoholics, and a trend in the same direction has also been observed in a study of a small sample of daughters (Erwin 1987; Lex and Mendelson 1987; Pollock *et al.*, 1986). The findings regarding subjective feelings were bolstered by the original reports of a decreased amount of change in body sway or static ataxia following drinking in our own and other laboratories (Schuckit 1985e; O'Malley and Maisto 1985), and these in turn have recently been corroborated among newer samples of both male and female children of alcoholic fathers (Lex and Mendelson 1987; Erwin 1977). In an effort to measure reactions to alcohol in more biological systems, earlier studies in our laboratory had documented lower intensities of change for prolactin and cortisol following drinking in FHPs (Schuckit *et al.*, 1983; Schuckit 1984b), findings which have been replicated more recently in a more demanding three session research paradigm (Schuckit *et al.*, in press a; Schuckit *et al.*, in press b). Thus, research presented over the last year continues to support a decreased intensity of reaction to alcohol in children of alcoholics as measured by subjective feelings, a motor performance test, and changes in hormones sensitive to ethanol. It is hypothesized that a decreased sensitivity to lower doses of alcohol might make it more difficult for some individuals at high risk for alcoholism to train themselves to stop drinking during an evening before they become too intoxicated to modify their behavior.

A second series of studies published prior to 1986 documented electrophysiological differences between FHPs and FHNs, including a decreased amplitude of the P3 wave of event-related potentials (ERPs) in alcoholics and in their sons (Begleiter *et al.*, 1984). Additional recent studies from the same laboratory (Begleiter 1987) and work using a similar research paradigm with college men have corroborated these results (Tasman *et al.*, 1986; O'Connor 1985), although Polich and colleagues have failed to replicate a decreased amplitude of P3's among sons of alcoholics using either an auditory or a visual ERP approach (Polich and Bloom in press; Polich *et al.*, in press). A second neurophysiological measure has involved the documentation in FHPs of a decreased amount of alpha power on background cortical electroencephalograms (EEGs) or a significantly enhanced increase in this wave following low doses of ethanol (Volavka *et al.*, 1982; Pollock *et al.*, 1983). Recent findings consistent with these results have been generated in those same laboratories (Pollock 1986), as well as through a collaboration between our group and Cindy Ehlers in a pilot study with 7 FHP/FHN pairs.

In a third area of research, sons of alcoholics have been reported to demonstrate higher levels of impairment on *neurocognitive testing* when compared to controls in studies generated both in the United States and Denmark (Schaeffer *et al.*, 1984; Knop *et al.*, 1985; Tarter *et al.*, 1984). While one recent investigation corroborated the earlier findings in 8 to 12 year old sons of alcoholics (Noble *et al.*, 1986), two large scale studies were unable to document any consistent cognitive deficiency in student and employed groups of men in their late teens to early 20's (Workman-Daniels and Hesselbrock 1987; Schuckit *et al.*, in press c). Thus, the question of the importance of neurocognitive differences between FHPs and FHNs is still unanswered.

A fourth area of research highlighted in reviews in 1985 is based on the observation that the *personality* profile of alcoholics may be unique, at least in the first months following detoxification. However, retrospective evaluation of personality test scores measured before the alcoholism developed did not reveal unusual characteristics (Kammeier *et al.*, 1973; Vaillant 1983; Vaillant 1984), and tests of men at high risk for alcoholism had revealed no significant differences from FHNs on anxiety, locus of control, the Eysenck Personality Inventory, the rod and frame test, nor on subtests of the MMPI (Saunders and Schuckit 1981; Schuckit 1982; Schuckit 1983 a; Tarter *et al.*, 1986; Schuckit and Penn 1985). More recent leads on personality variables include the possible importance of a scale from the MMPI modified specifically for adolescents, and an additional scale evaluating novelty-seeking, harm avoidance and reward-dependence (Cloninger 1987; MacAndrew 1986; DeWit *et al.*, 1987). The current studies however, have found no evidence that FHPs have an increased tendency toward sensation seeking or type A personalities, and tend to question the importance of any major personality variable (Beardslee *et al.*, 1986; Manning 1986). Similar to neurocognitive results, more work will be required before the role of personality profiles in the alcoholism risk can be understood.

Several additional laboratories have recently evaluated *other possible markers*. Regarding the intensity of reaction to alcohol, Erwin and colleagues have raised the question of a possible greater drop in beta-endorphin in the plasma following alcohol in sons of alcoholics (Erwin 1987), while Linnoila and colleagues have reported differences between sons of alcoholics and controls in their thyroid stimulating hormone (TSH) response to thyrotropin releasing hormone (TRH) infusions (George *et al.*, 1986; Linnoila 1985). An additional recent electrophysiological measure of relevance is Linnoila's observation of less change in saccadic eye movement after a benzodiazepine for sons of alcoholics (Linnoila 1985), as well as the documentation by Newlin and

Aldrich (1986) of differences between 9 FHP and 9 FNH men on the autonomic response observed in their second ethanol challenge and on the intensity of reaction to placebo.

An additional current development centers on the simultaneous evaluation of multiple attributes of FHPs and FHNs. Until recently, most studies have focused on only one type of finding at a time, rarely including a large enough sample to evaluate interactions between subjective feelings, cognitive test performance and hormonal changes. Our laboratory has just completed a series of baseline and post-challenge evaluations following placebo, 0.75 ml/kg of ethanol, and 1.1 ml/kg of ethanol in 30 sons of alcoholics and 30 controls (60 men) (Schuckit and Gold, in press). Using a discriminant function analysis, four items (the maximum terrible subjective feelings after high dose, cortisol values at two time points after high dose, and a prolactin result after low dose) combined to correctly identify 83% of the controls and 70% of the sons of alcoholics. This included approximately 40% of each group whose discriminant scores were +1 or -1 and who were considered to be solid classifications. These results were relatively robust on a jackknife validation procedure. A search for clusters of factors of test scores after ethanol using a principal components analysis was also consistent with the discriminant analysis. The PCA indicated the possibility of three overlapping domains of the ethanol response including subjective feelings after the high dose ethanol challenge (explaining 46% of the variance); hormonal changes after low dose ethanol as well as body sway items (14 percent of the variance); and prolactin changes after low dose ethanol (9% of the variance).

SOME CONCLUSIONS

Impressive progress has been made in the last decade regarding our knowledge of genetic factors relevant to alcoholism. Studies have progressed from questioning whether alcoholism is genetically influenced to attempting to increase our understanding of biological and genetically mediated factors that might contribute to the alcoholism risk. Recognizing that a number of recent reviews have throughly discussed the importance of genetic factors and have presented a number of methodological issues relevant to studying populations at high risk for this disorder, this paper has focussed on several additional methodological questions and reviewed some very recent results.

Reflecting our lack of sophisticated measures of genetic influences and difficulties in accurately diagnosing alcoholics, it is important to keep studies of populations at high risk as narrowly focused as possible. Thus, while there is crossover in symptomatology between alcoholism, depressive disorders, and antisocial personality disorder, most studies have centered on children of carefully defined primary alcoholics. Evaluations of such individuals from preteen years to early adulthood have documented numerous leads for potential FHP/FHN differences that might relate to the alcoholism risk, although no specific, sensitive, and reliable marker (e.g., a protein) has yet been identified. The most important and consistent leads include a decreased intensity of reaction to alcohol (a behavioral observation), a decreased amplitude of the P3 brain wave of the event-related potential, and a decreased amount of alpha power or a greater increase in the amount of this wave after ethanol for sons of alcoholics. Additional investigations are beginning to evaluate the generalizability of the major findings, and an effort is being made to simultaneously evaluate multiple aspects of baseline and post-ethanol responses.

A number of additional steps are required to allow for optimal progress. First, it is hoped that the various laboratories evaluating populations at high risk for alcoholism will begin to standardize their definitions and methods for dealing with the relationship between alcoholism and other genetically influenced disorders. Second, it is hoped that laboratories can begin to standardize the baseline and post-beverage challenge procedures so that greater understanding of the variability and generalizability of test results will ensue. Finally, recognizing the great expense involved in identifying, testing, and following populations at high risk, it is hoped that major funding agencies will be able to generate additional long-term commitments to these studies.

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Progress in Understanding the Conditioning Aspects of Drug Dependence

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INTRODUCTION

The puzzle of addiction has confounded clinicians and researchers for decades. Many theories have been invoked to explain the *initiation* of drug use and the *continuation* of drug use, but the problem of *relapse* has remained difficult to explain. Why is it that after months or even years of abstinence, a former addict may again lose control and resume drug use when exposed to certain situations? Is there some retained susceptibility to addiction in a drug-free former addict who feels physically well and expresses strong commitment to refrain from future use?

One of the first theorists to address this question was Abraham Wikler (1948). At the Addiction Research Center in Lexington, Kentucky, in the 1940's he began to consider the possibility that relapse in recovering addicts might be influenced by classically conditioned responses. Wikler was aware of work by Pavlov and colleagues (1927) demonstrating that repeated injections of morphine could produce a form of learning in dogs. Subsequently the mere sight of the investigator preparing to give the injection could produce a response in the animal which resembled morphine effects (e.g. salivation, vomiting and sedation).

Wikler suggested that some of the behaviors observed in addicts might also be examples of Pavlovian conditioning. He observed that during group therapy sessions, the discussion would often turn to the details of drug procurement and usage. During such times when the topic of drugs was being discussed, the *drug-free* recovering addicts showed yawning and tearing as though they might be going through mild withdrawal.

A similar behavior which Wikler noted was the report of withdrawal symptoms in drug-free recovering addicts upon their return home. Typically the former addict felt well just after leaving the hospital, however, on returning to his home neighborhood, symptoms similar to withdrawal sickness began to occur. Sometimes there would be actual nausea and vomiting as well as the strong urge to inject heroin during this period just after leaving the hospital. The recovering addict would often give in to this craving and quickly become re-addicted.

These clinical observations led Wikler to begin a series of laboratory studies with animals confirming that withdrawal symptoms could be classically conditioned (Wikler and Pescor, 1967). He showed that a distinctive environment repeatedly paired with morphine withdrawal in rats could eventually acquire the ability to evoke signs of withdrawal after the rats were healthy and drug-free. This work was confirmed by other investigators over the years in other species and analogous results were obtained using other drugs including alcohol, stimulants and haloperidol. This literature has been extensively reviewed (e.g. Grabowski and O'Brien 1981).

On the basis of his clinical observations and animal studies, Wikler postulated that repeated drug self-administration produces a new disease *suis generis* (of its own kind). This new disease consists of the conditioned responses produced by pairing the effects of drugs (or withdrawal) with environmental or internal stimuli. In recent years our group has attempted to test and expand this hypothesis using an experimental approach in several different clinical populations.

CATEGORIES OF CONDITIONED RESPONSES

Studies in human subjects show that both drug-like and drug-opposite responses can be produced in human subjects as conditioned responses (CR's) depending on the circumstances (For reviews, see O'Brien et al., 1986; O'Brien et al.,1987). We have attempted to classify these responses according to the proposed mechanism of their origin and the conditions under which they can be demonstrated.

Drug-opposite CR's
  a. Conditioned withdrawal
  b. Conditioned tolerance

Drug-like CR's
  a. Conditioned euphoria ("Needle-Freak" phenomena)
  b. ? Placebo effects

DRUG-OPPOSITE CONDITIONED RESPONSES

Drug-opposite conditioned responses are those responses which are elicited by previously neutral stimuli (CS's) following a series of pairings with a drug (Unconditioned Stimulus, US), but which are opposite to the responses produced by the drug itself. For example, opiate injections produce *elevations* in skin temperature, but stimuli which have preceded opiate injections will reliably produce *reductions* in skin temperature. There are many other examples of "drug-opposite" CR's which can be measured by polygraphic measurement of physiological changes, ratings by patients of subjective changes or observer ratings of patient behavior.

Conditioned withdrawal was the first type of conditioning considered by Wikler and it still is the phenomenon most closely associated with his name. Actually, Wikler studied other types of conditioning phenomena and he did not view dependence as simply avoidance of withdrawal. The presumed mechanism for the development of conditioned withdrawal is shown in Figure 1. Since some withdrawal symptoms may occur at least once per day in most opioid addicts, there may be thousands of pairings of environmental stimuli with these withdrawal symptoms during the life of a patient before he seeks treatment. We have shown that after as few as seven pairings of mild withdrawal symptoms and a novel conditioning stimulus (CS), humans begin to show signs of withdrawal (Conditioned Response) when exposed to the CS alone (O'Brien et al 1977). These CR's have been found to be long-lasting (Goldberg and Schuster 1970) and they have been found to occur when the subject is exposed to the CS long after detoxification from drugs. This mechanism could, therefore, explain the stories reported by Wikler (1965) and others (O'Brien 1975) concerning onset of withdrawal symptoms when a drug-free patient returns to an area where withdrawal symptoms had occurred in the past.

FIGURE 1

Conditioned Withdrawal (Dependent Subject)

Unconditioned Stimulus *Opioid metabolized* *Receptors evacuated*	------>	**Unconditioned Response** *Rebound activity; adrenergic, cholinergic, etc. (tearing, rhinorrhea, tachycardia, nausea, diarrhea, etc.) Drug-opposite effects*
Conditioning Stimulus *Drug -procuring or -using environment: sights, sounds, smells, situations, fantasies*	------>	**Conditioned Response** *mild version of above symptoms, drug-opposite effects*

Conditioned tolerance is a term applied to another mechanism whereby drug-opposite responses might be conditioned. Siegel, in a series of experiments utilizing morphine, alcohol and insulin (see Siegel 1978, 1979, 1987), presented evidence that drug tolerance could be considered, at least in part, to be a classically-conditioned phenomenon. As shown in Figure 2, the drug disturbs homeostatic equilibrium resulting in a reflex response against the drug as the organism attempts to regain equilibrium. This reflex response (Unconditioned Response) tends to reduce the effects of the drug. The environmental cues (sights, smells, situations) repeatedly associated with drug procurement or injection provide a signal (CS) which, after some repetition, can by themselves trigger homeostatic responses (tolerance) which are opposite to drug effects. The conditioning of tolerance was termed "counter-adaptation" by Wikler (1953, p. 44).

FIGURE 2

Conditioned Tolerance (Dependent or Non-Dependent Subject)

Unconditioned Stimulus *Drug injection, Drug effects, Disturbances in homeostasis produced by the drug*	——>	**Unconditioned Response** *Homeostatic response counter to drug effect so as to return to status before the drug (TOLERANCE)*
Conditioning Stimulus *Sights, sounds, smells which signal that drug is about to appear*	——>	**Conditioned Response** *Homeostatic responses counter to drug effects which in the absence of drug can be perceived as WITHDRAWAL or ?CRAVING*

These conditioned drug-opposite or tolerance responses can occur in a drug user who has never been dependent on drugs. but has only used intermittently. Also, repeated episodes of withdrawal sickness in a specific environment would not be necessary for the development of drug-opposite responses according to this theoretical mechanism. However, the CR is physiologically similar to a withdrawal response and therefore the symptoms may be perceived as "withdrawal-like." Those drug users who *have* had repeated episodes of withdrawal in a specific environment will thus have *two mechanisms* for producing conditioned withdrawal-like symptoms: the first by the "conditioned withdrawal" paradigm described in Figure 1 and the second by the "conditioned tolerance" mechanism described in Figure 2 .

DRUG-LIKE CONDITIONED RESPONSES

Pavlov's original report (1927) of morphine conditioning described a CR which resembled the unconditioned effects of morphine itself. Similar findings of "drug-like" conditioning have been reported by others in dogs (Collins & Tatum 1925; Lynch et al.,1976; Rush et al.,1970) and in rats (Eikelboom & Stewart, 1979; Miksic et al.,1975; Numan et al.,1975).

Drug-like effects are found clinically in patients known as "needle freaks" (Levine 1974). Typically these are individuals who may formerly have been physically dependent on opioids, but are currently using drugs intermittently or using low potency drug supplies. These "needle freaks" report *euphoria* from the act of self-injection and they have also been observed to show physiological signs such as pupillary constriction after injecting saline (O'Brien 1975). A similar finding was reported by Myer and Mirin's (1979). Some of these "needle freaks" have been detected among applicants applying for methadone treatment. Federal regulations limit the use of methadone maintenance (except in certain special cases) to individuals who are physically dependent on opioids. If there are no signs of withdrawal in an applicant, the opioid antagonist naloxone may be given as a diagnostic test for the presence of dependence (Blachly 1973). Even a very small dose of naloxone will precipitate withdrawal symptoms in a person physically dependent on opioids. Occasionally we have observed the naloxone injection to produce *mild euphoria instead of withdrawal* in applicants for methadone who claim to be addicts and who show the scars of chronic drug injections. Subsequently we observed sedation and reports of euphoria when these subjects self-injected saline; thus the euphoria observed after naloxone was not a pharmacological effect of naloxone but likely a conditioned response to the injection procedure which served as a conditioned stimulus (O'Brien 1975).

There have been few direct observations using physiological and psychological monitoring of human addicts in the act of self-injecting addicting drugs. Our group reported a series of such studies (O'Brien 1975; O'Brien et al.,1974; O'Brien et al.,1980) that described self-injections in detoxified opioid addicts being treated with the opioid antagonists cyclazocine or naltrexone. Several experimental protocols were used with these antagonists which block the pharmacological effects of opioids. In one series of experiments, the patients were randomly assigned to self-injections with either saline or opioid; in others the patients were tested with both saline and opioid on different occasions. These experiments began with "credibility" trials in which the subject was allowed to self-inject opioid or saline (double-blind, unblocked) prior to beginning antagonist maintenance. Subsequently we conducted "extinction trials" in which the subject repeatedly self-injected opioid or saline while being maintained on the opioid antagonist for up to 6 months. The findings were that saline self-injections were usually reported as pleasurable and identified as a low dose of opioid. This reaction to saline was assumed to be a drug-like conditioned response. The effect was greatest when the subjects injected themselves under naturalistic conditions resembling the patient's "shooting gallery" with the patient *expecting* to get "high." The drug-like effect was diminished but still present when the patient was placed alone in a more artificial setting, such as a recording chamber, with various electrodes and strain gauges attached.

We found that these drug-like effects in most patients were not long-lasting when compared to the drug-opposite effects described above. After several un-reinforced trials consisting of either saline injections or opioid injections in patients pre-treated with a narcotic antagonist, the drug-like effects disappeared. The drug-opposite effects persisted in these patients, however.

Meyer and Mirin (1979) used a different design and also observed conditioned opioid-like autonomic effects in human subjects. Their subjects were all recently detoxified inpatients who were given either naltrexone or naltrexone placebo under double-blind conditions. The subjects were then permitted to self-inject known amounts of heroin that they had earned by performing a simple operant task. The subjects who received naltrexone placebo in effect had the opportunity to inject " free" heroin unimpeded by naltrexone, and they injected it nearly the maximum number of times permitted by the protocol. However, the 22 subjects who received naltrexone had the rewarding effects of heroin blocked by this antagonist. Eleven of these subjects stopped injecting heroin after fewer than five trials, but the other 11 subjects took an average of 16 doses of heroin despite the presence of naltrexone. These 11 subjects were found to be different from those who stopped quickly in that they showed distinct autonomic changes resembling opioid effects after the first three presumably blocked injections. The authors interpreted these autonomic changes (pupil, heart rate, and blood pressure) as conditioned opioid-like effects and they found that these autonomic changes had disappeared (extinguished) by the time the subjects decided to stop injecting. Unlike the outpatient studies described above, the Meyer and Mirin protocol did not require the subjects to continue to inject unless they wished to do so. Since they did not continue injecting past the point at which the patient's response to the procedure changed from positive to neutral, this probably explains why unpleasant or withdrawal-like symptoms were not reported.

Thus,the evidence for conditioned opioid-like effects in humans is based on clinical anecdotes and the self-injection studies described above. These CR's are elicited by the complex CS of pre-injection rituals and the act of self-injection. In most subjects the opioid-like CR is extinguished quickly and then withdrawal-like CRs are elicited by the same CSs that previously produced opioid-like effects.

CLINICAL RELEVANCE OF CONDITIONING PHENOMENA

Now that there is laboratory evidence that opioid drugs can produce conditioned responses in humans as well as animals, it is proper to ask whether there is any clinical relevance to these phenomena. We developed a set of standard drug-related stimuli consisting of video tapes and paraphernalia associated with the procurement and injection of opioids. When we presented these drug-related stimuli to patients while measuring their behavioral, subjective and physiological responses (Childress et al 1986), the most common responses were increases in feelings of drug "craving" with or without evidence of conditioned withdrawal. Reports of drug-like effects were less common. Presumably, the patients would have reacted even more strongly to stimuli from their own environment as contrasted to the standard set.

Additional research in this area indicates that these conditioned responses are long-lasting and remain robust even after the completion of 30 days of drug-free addiction treatment (Childress et al 1987). In these studies, formerly dependent opiate addicts were tested with our standard stimuli following completion of a 30-day therapeutic community treatment program. Thirty-six per-cent of these patients showed pronounced drug-opposite changes in their physiological responses (usually skin temperature and skin resistance) and 90 % reported subjective increases in feelings of withdrawal and craving. The majority of these patients were surprised and upset by their responses. Several suggested that "...it's as though I was never in treatment..."

While the majority of work in this laboratory has focussed on opioid addiction, we have recently completed a similar series of studies with cocaine-dependent subjects, showing very similar results. In our laboratory, sets of cocaine-related video tapes, audio tapes and paraphernalia reliably elicit physiological arousal and strong subjective reports of craving and withdrawal. Again, these responses were seen in patients even after 30 days of therapeutic community treatment (Childress et al 1987, in preparation). These findings support Wikler's original concept of a "new disease," *suis generis* and suggest that treatments which focus exclusively on the physiological aspect of addiction and ignore the learned or conditioned aspects may not be providing adequate care.

Wikler's description of conditioned abstinence or withdrawal caused clinicians to begin looking for such phenomena in their patients. Relapse occurs in the vast majority of detoxified patients and when asked about reasons for relapse, most patients report very little insight into their own behavior. The first drug use after a detoxification usually appears to be an impulsive act. The impulsivity can be so marked that the individual often really does not know why he performed a given action. When questioned about the reasons for a particular drug administration, the typical response is, "I wanted to get high." Addicts usually do not talk spontaneously about relieving withdrawal or discomfort. However, tolerance tends to diminish drug pharmacological effects and street drugs are notoriously low in purity; therefore, the injection of opioids often simply relieves withdrawal and does not produce the desired euphoria.

McAuliffe (1982) interviewed 40 street heroin addicts in an attempt to assess the role of conditioned withdrawal in relapse. All of these subjects had experienced a period of abstinence on the street. While 27.5% reported being aware of conditioned withdrawal, only two (5%) gave this as a reason for resuming drug use. The author concluded that conditionied withdrawal was unlikely to be a major cause of relapse. Of course, it is difficult to extrapolate self-report data from active street addicts and apply them to the problem of relapse after treatment.

Our group also conducted interview studies with former addicts trying to remain drug-free and found varied reasons for relapse. A third to a half of the subjects were able to identify places or situations that made them feel unexplainably ill, anxious, or in need of a fix (Childress et al 1986c; O'Brien, 1975). Of particular interest was that certain moods (e.g., depression or anger) were found to trigger drug craving or sickness (Childress et al 1987). In these reports, negative feelings, affects, or even withdrawal sickness (sniffing, tearing) were commonly mentioned, but euphoria was rarely experienced as a possible conditioned effect. Our patients were able to recall situations or places where they began to feel a desire (craving) or need for an injection, but they rarely reported the opposite feelings, that is, situations that made them feel euphoric in the absence of a drug.

Surveys which rely on patients' memories of past experiences are subject to many sources of potential error. In order to improve our source of information, we conducted an eight week *prospective* study which involved weekly structured interviews concerning situations which provoked desires to use drugs. Seventeen methadone patients have been studied in this manner and interviews of abstinent, former opioid abusers are now in progress. The structured interviews enabled us to obtain a clearer picture of the occurrence of drug-related responses over time. Sixteen of the seventeen methadone patients (94%) reported episodes of drug craving in real-life situations (e.g. sight of a drug-using friend) which occurred during the period of study. Sixteen of seventeen patients (94%) also experienced episodes of withdrawal-like feelings. Though patients often attributed these withdrawal-like episodes to physical discomfort or "methadone-dose- -not-holding," some of these episodes may have been conditioned in origin: their occurrence was *unrelated* to the time since the last dose of methadone. Spontaneous high-like feelings were less commonly reported, but 76% of the methadone patients reported at least one such episode (Childress et al l986a).

Among the abstinent patients who have been followed for eight weeks, *all* (8 of 8 thus far) have reported episodes of opioid craving, averaging 11 episodes *per patient* over the eight week period. In contrast to the frequency of craving episodes, reports of withdrawal and high-like episodes were relatively rare (totalling 3 and 2 episodes respectively). These results are still being analyzed and are being used to develop a database on the sequence of events in *relapse*. We are in the process of collecting the same type of "natural incidence" data from abstinent cocaine abusers as they proceed through outpatient follow-up. Our longitudinal work in this area thus far, indicates that the concurrent collection of data from outpatients is feasible. By this longitudinal method, we hope to obtain a clearer and perhaps more valid picture of the role of conditioning in relapse to drug dependence.

POSSIBLE INTERVENTIONS BASED ON CONDITIONING

The most important benefit of a theory of drug dependence is the potential practical value. Does the theory lead to a new form of prevention or treatment for this enormous public health problem? Relapse to drug abuse is such a complex problem that changing a single factor may not significantly influence the outcome. We have attempted to reduce or eliminate the conditioned responses found in drug abusers by a process called *extinction*. This involves repeatedly showing the patient the drug-related stimuli which produce symptoms, usually craving or withdrawal symptoms. Our first attempt at extinction involved having the drug-free recovering heroin addict perform un-reinforced self-injections (either saline or opioid blocked by antagonist) (O'Brien et al 1979). This procedure was impractical because it produced such severe conditioned responses. We now expose the patient to drug-related stimuli in a more gradual way beginning with stimuli remote from the act of drug administration. We also combine the extinction with a comprehensive rehabilitation program which involves attention to psychiatric disorders, family problems, employment problems-- in short, attention to conditioned responses becomes just one more aspect in a multi-modality approach to treatment.

Using this multi-modal approach, recent work (Childress et al 1986, 1987) shows that in both former cocaine-dependent and former opioid-dependent patients, the extinction procedure produces a marked diminution of the conditioned responses over 15 to 30 sessions. There is also a significant improvement in the majority of cases, but few remain drug-free. Several important questions remain:

a. Is the extinction procedure truly effective in reducing conditioned responses? We see decreases in the patient's responses while in the laboratory, but is there any generalization to the patient's "real world?" Perhaps we need more personalized stimuli for extinction; perhaps we must deal with the difficult problem of going into the patient's environment and continuing the extinction where relapse is most likey to be precipitated.

b. Even if we are able to successfully extinguish the conditioned responses, will this have any effect on relapse? All of the conditions which caused the patient to use drugs the first time are probably still present after treatment. Obviously some relapses have nothing to do with conditioning. Only prospective studies with random assignment and adequate sample size will show whether there is a subset of patients who *want* to avoid resumption of drug use and whose chances will be improved by eliminating the conditioning factor.

c. Are conditioned responses simply *epiphenomena* which occur in association with drug use, but have no role in determining resumption of drug use? Clearly this is a logical possibility which can be addressed by appropriate controlled studies of treatment outcome.

Finally, one must consider the problem of reinstatement of conditioned responses. If during the drug-free period, the patient "slips" for any reason and uses drugs again, the conditioning responses are likely to be rapidly reinstated. Thus a long and difficult period of extinction could be quickly neutralized. These thoughts are sobering, but realistic. We have progressed a great deal in understanding what conditioned responses are likey to occur under which conditions. This understanding of conditioning is interwoven in many of the current approaches to the prevention of relapse in all types of substance abusers. But we still lack evidence that either conditioning factors or any other element of modern treatment programs are actually effective in preventing relapse.

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Due to space limitations, references are not included but are available on request.

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Psychiatric Diagnoses and Substance Abuse in the General Population: The ECA Data

J. Helzer

The ECA Survey

The Epidemiologic Catchment Area (ECA) survey (Regier et al., 1984) is a multicenter study of the population prevalence and incidence of psychiatric disorder and associated health utilization in the United States. During the course of this investigation, approximately 20,000 persons were personally interviewed to obtain baseline information on the lifetime and recent occurrence of psychiatric symptoms and disorders. The baseline examination was then followed by at least one telephone interview several months later and at least one follow-up personal interview one year later, to ascertain more recent use of services, and new occurrences of illness in order to estimate annual incidence of psychiatric disorder.

In addition to information about the prevalence and incidence of psychiatric illness, the ECA also ascertained data on associated risk factors, treatment seeking, barriers to treatment, and other issues related to psychiatric impairment. Furthermore, the survey will provide baseline samples which can be followed over time in order to help fill our tremendous gaps in knowledge about the natural history of mental illness and substance abuse in the general population.

The ECA is neither a random sample of the entire United States, samples were drawn from 5 cities only, nor does the Diagnostic Interview Schedule (DIS) (Robins et al., 1981), the interview instrument used, cover the entire range of DSM-III disorders (American Psychiatric Association 1980). But the major disorders are covered and the data presented in this report have been combined from the five ECA sites and weighted to match the age/sex/race distribution of the total U.S. population.

For the interpretation of the findings reported here, it is important to understand that the DIS is based on lifetime rather than cross-sectional ascertainment of symptoms and illnesses. The interview ascertains whether each symptom in a particular diagnostic set has ever occurred and the ages of first and most recent symptoms, representing ages of onset and recency, respectively. For some disorders, like the affective disorders, the diagnosis is based on the occurrence of a simultaneous cluster of symptoms, because that's what DSM-III specifies. But for others, like the substance abuse disorders, there is no clustering requirement. Thus the minimum of two alcohol symptoms specified by the DSM-III may be separated by a period of several years and there is no guarantee there was ever a cluster of symptoms or alcohol problems occurring together.

Prevalence Findings

Table 1 shows the lifetime prevalence estimates for the major psychiatric disorders as ascertained in the ECA survey (Eaton and Kessler 1985). We have collapsed individual diagnoses (specific drugs of abuse, for example) into single categories (i.e., drug abuse and/or dependence). Since the disorders are presented here nonhierarchically, a given individual may fall into more than one category.

TABLE 1. Lifetime prevalence of major DSM-III disorders in five cities (ECA 5-site data, weighted to the U.S. population) (Unweighted N = 19,357)

Disorder	Lifetime Prevalence
Alcohol abuse and/or dependence	13.7
Phobic disorder	12.6
Drug abuse and/or dependence	5.9
Major depression	5.1
Antisocial personality	2.5
Obsessive compulsive disorder	2.5
Dysthymia	1.5
Panic disorder	1.5
Cognitive dysfunction	1.1
Schizophrenia	1.0
Mania	0.4
Somatization disorder	0.1
Anorexia	0.1

According to these findings, alcohol abuse and/or dependence (also for convenience called alcoholism) is the most prevalent of the psychiatric disorders we examined. It is important to recognize that since the data are based on lifetime occurrence of symptoms, the diagnosis here may be closer to what some might consider alcohol problems rather than alcoholism. DSM-III requires one or more alcohol related problems in each of two symptom categories for the diagnosis of abuse. For alcohol dependence, the requirement is a minimum of one alcohol problem and evidence of tolerance to alcohol or withdrawal symptoms.

Examining the rest of the table, the substance use disorders are seen to figure very prominently; drug abuse and/or dependence is the third most prevalent disorder. Only phobic disorder rivals alcoholism in terms of prevalence, and drug abuse/dependence is slightly more prevalent than major depression. Antisocial personality disorder, another diagnosis that will figure prominently in this report, is about half the lifetime prevalence of major depression.

Data in Table 1 are aggregated across the five ECA sites and, as mentioned above, the sample has been adjusted to the age/sex/race distribution of the U.S. population as of the 1980 census. Figure 1 shows that drinking patterns are not uniform across the five sites. The first category shown in Figure 1 is those who have never taken a drink of alcohol, i.e. lifelong, total abstention. Social drinkers are those who deny total abstention and also deny either a period of heavy alcohol consumption and or any of the alcohol-related problems we asked about. Heavy drinkers also deny lifetime problems but report consumption of seven or more drinks at least one evening a week for a period of several weeks or more at some time in their lives. Problem drinkers report one or more lifetime drinking problems, but fail to meet the full criteria for abuse or dependence. Heavy and problem drinkers are aggregated in the figure. The final category is those who do meet the full DSM-III criteria for alcoholism.

In terms of heavy/problem drinking and alcoholism rates, the sites fall into two groups: St. Louis, Baltimore, and Los Angeles, where the rates are relatively high, and Durham and New Haven, where they are relatively lower. Apart from this similarity, the drinking patterns at the latter two sites are very different. New Haven has the lowest rate of abstinence, and the highest rate of social drinking. For Durham, a more rural region lying in the Bible Belt, the opposite is the case.

Comorbidity

Next we examine the comorbidity of alcoholism with other psychiatric disorders, including the abuse of other substances. Thirty four percent of the total ECA sample met lifetime criteria for at least one of the diagnoses shown in Table 1. Of those 34%, about an equal proportion (32%) had a second core diagnosis. As we saw above, about 13% of the total sample had a diagnosis of alcohol abuse or dependence, but the odds of having a second diagnosis in this group is almost half (47%). Thus alcoholics are very much more likely than the total ill sample to have met criteria for at least one other psychiatric disorder. Figure 2 shows that a good bit of this comorbidity is accounted for by drug abuse and dependence, including both canabinoids and harder drugs. Among those with no diagnosis of alcohol abuse or dependence, 3.5% have a lifetime history of any illicit drug use sufficient to get a diagnosis of drug abuse or dependence, and most of this reported drug use is marijuana or some other canabinoid (Figure 2). Among those with an alcohol diagnosis, the lifetime prevalence of drug abuse or dependence is much greater and half of this drug abuse is of harder drugs, compared to a much smaller proportion of hard drug use in the nonalconolics.

Figure 2 can be contrasted with Table 2, the latter showing the lifetime history of alcohol abuse or dependence among those abusing drugs. Recall (from Table 1) that the population rate of alcoholism is over 13%. As shown in Table 2, this lifetime prevalence is only 11% among those who have never abused drugs. This rate is considerably higher (36%) in those who have a history of marijuana abuse but no other drugs. Among those who have abused or been dependent on harder drugs, the lifetime prevalence of alcoholism is over half (55%).

TABLE 2. The association of alcohol and drug abuse or dependence (5-site ECA data weighted to U.S. population)

Group	Lifetime Prevalence of Alcoholism (%)
Total sample	13.7
Respondents with lifetime history of:	
No drugs	11
THC only	36
Sedatives, hypnotics, or opiates	55

Although this association is striking, comorbidity with alcoholism is not confined to other substances of abuse, as is illustrated in Figure 3. This shows the weighted lifetime prevalence of the major, nonalcohol diagnoses in the total ECA population, by sex, on the left side of the graph. On the right side, these same lifetime prevalences are shown for respondents with alcohol abuse and/or dependence. First it can be seen that the prevalence rates rise for both sexes for every diagnosis.

There are some other noteworthy findings as well. First, for none of the diagnoses is the change in prevalence from the total to the alcohol sample trivial. Major depression is not greatly increased in alcoholic men compared to all men, but the prevalence does almost double (3% to 5%). Among women this prevalence almost trebles (7% to 19%). These differences are even greater for other diagnoses. For example, antisocial personality disorder is almost 4 times greater than the population rate among alcoholic men, and almost 10 times greater in alcoholic women.

Another noteworthy finding in Figure 3 is that while some disorders are male predominant in the total population, all are female predominant in the alcoholic population. Even the male:female ratio for drug abuse/dependence, which is 1.4/1 in the general population, is 1/1.6 among alcoholics. Since it is so much more deviant for women to be alcoholic than for men, it is not surprising that the association of alcoholism with other diagnoses is stronger in women.

Conclusion

This has been a brief examination of the comorbidity between alcohol abuse/dependence and other substance and nonsubstance psychiatric diagnoses. Other aspects of these associations in the ECA data have also been examined in a recent article in the periodical literature (Helzer and Przybeck in press), and the interested reader is referred there for more detail. It is clear, however, from this brief report that alcoholism is associated with all of the other major psychiatric disorders, even in a general population sample in which one might expect less comorbidity. The strongest associations were with antisocial personality disorder and, not surprisingly, other substances of abuse. There was a weaker association between alcoholism and depression, which, in fact, tends to be contrary to reports from clinical samples (Hesselbrock et al., 1985). However, this is probably due to the difference in samples. It is logical to assume that one of the factors which might motivate alcoholics to seek treatment would be the occurrence of major depression. If that is the case, the comorbidity between these two would indeed appear greater in subjects drawn from a treatment setting compared to a population sample unselected for treatment seeking. Furthermore, as we showed in Figure 3, the association between alcoholism and major depression is a good bit stronger for women than it is for men.

These results have important implications for the recognition and the treatment of alcoholism. First, it seems clear that clinicians are underattentive to the existence of alcohol problems. In our own review of over 1200 inpatient and outpatient records, we frequently found records in which an occasional symptom was mentioned, but no evidence of a systematic review of alcohol problems could be found (Helzer et al., 1985). Others have provided evidence that alcoholism is underrecognized in clinical settings (Coulehan et al., 1987). By increasing the likelihood that alcoholics will come to treatment, comorbidity with other disorders increases our opportunities to detect alcoholism and thus to offer appropriate care to those who might not otherwise be receiving it. On the other hand, the potential opportunity for increased recognition that comorbidity provides might be lost if attention is focused exclusively on the comorbid diagnosis, or if the overlapping of two disorders produces a confusing clinical picture. This argues strongly for further attention to physician education, both in terms of increasing the provider's alertness to the possibility of alcoholism in the context of other illness and of accurate recognition when such "double diagnosis" occurs (Moen and Batey 1986).

There are also important implications of the findings here for the appropriate treatment of alcoholism. The high degree of comorbidity between alcoholism and other disorders in the community argues for increased research attention to the natural history and the treatment of comorbid disorders. Research on the natural history of alcoholism per se in the general population is badly needed (Polich and Kaelber 1985). But if, as the findings here suggest, half of the alcoholics in the general community have a second psychiatric diagnosis, our research agenda for this most prevalent of psychiatric disorders is even further behind than we suspected.

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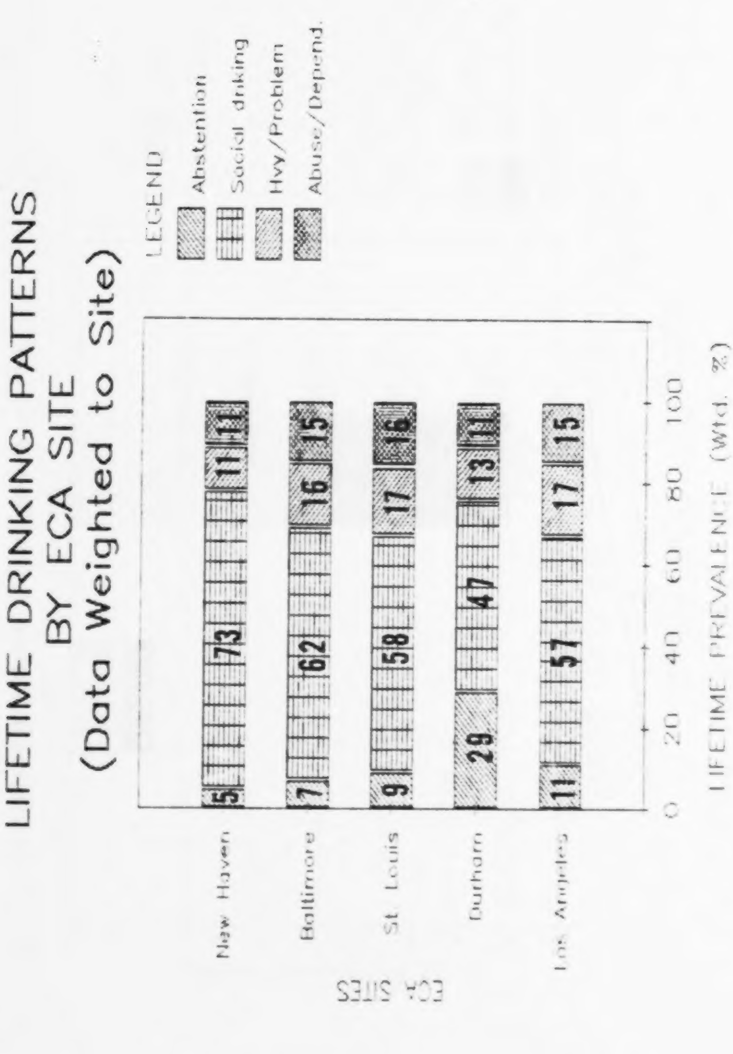
LIFETIME DRINKING PATTERNS
BY ECA SITE
(Data Weighted to Site)
LEGEND
▨ Abstention
▥ Social drinking
▧ Hvy/Problem
▩ Abuse/Depend.
New Haven
5
73
11
11
Baltimore
7
62
16
15
St Louis
9
58
17
16
Durham
29
47
13
11
Los Angeles
11
57
17
15
ECA SITES
0
20
40
60
80
100
LIFETIME PREVALENCE (Wtd. %)

FIGURE 1

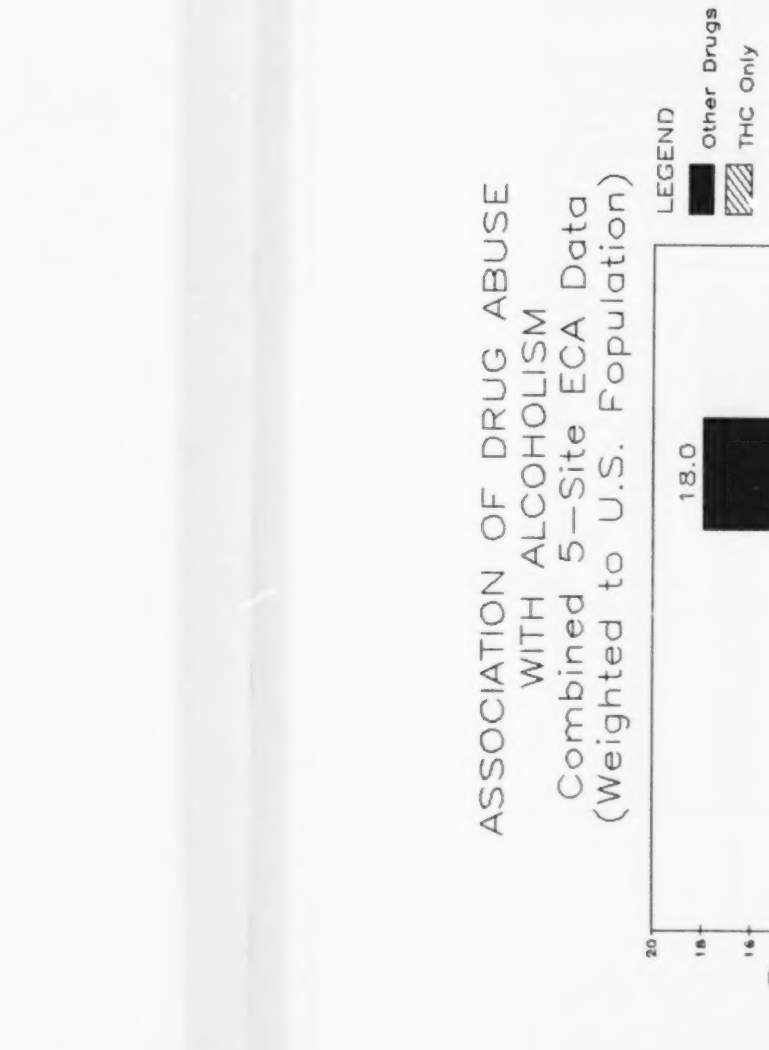
ASSOCIATION OF DRUG ABUSE
WITH ALCOHOLISM
Combined 5–Site ECA Data
(Weighted to U.S. Population)
LEGEND
Other Drugs
THC Only
Lifetime Prevalence (wtd. %)
20
18
16
14
12
10
8
6
4
2
0
3.5
18.0
NO
YES
Alcohol Abuse &/or Dependence

FIGURE 2

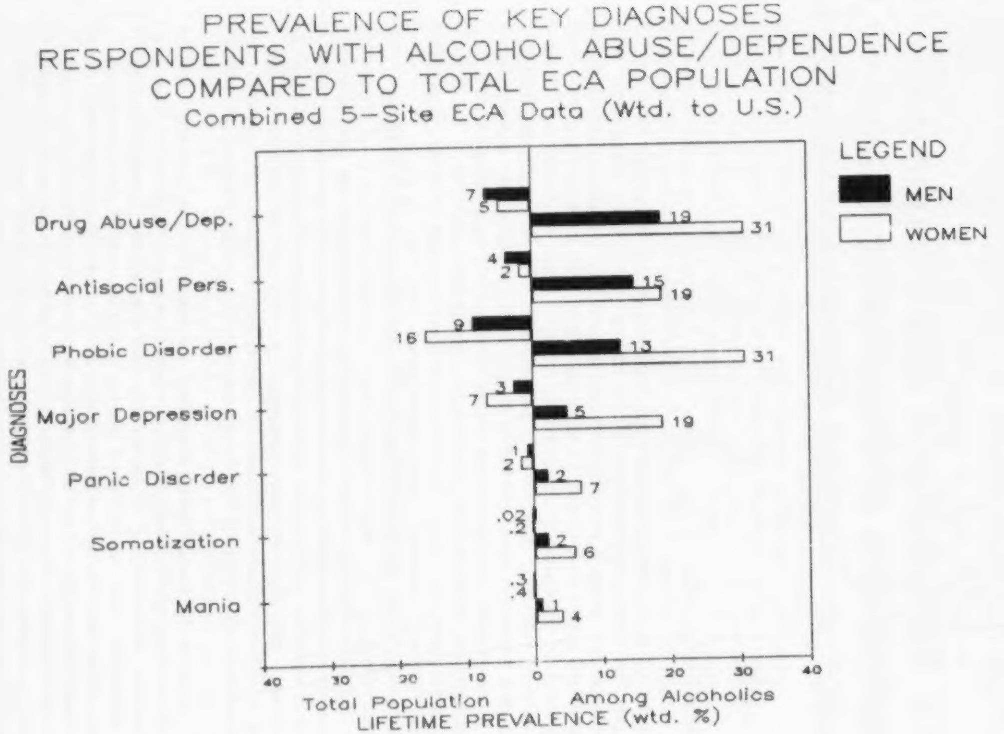
PREVALENCE OF KEY DIAGNOSES
RESPONDENTS WITH ALCOHOL ABUSE/DEPENDENCE
COMPARED TO TOTAL ECA POPULATION
Combined 5-Site ECA Data (Wtd. to U.S.)
LEGEND
MEN
WOMEN
DIAGNOSES
Drug Abuse/Dep.
Antisocial Pers.
Phobic Disorder
Major Depression
Panic Disorder
Somatization
Mania
7
5
19
31
4
2
15
19
9
16
13
31
3
7
5
19
1
2
2
7
.02
.2
2
6
.3
.4
1
4
40
30
20
10
0
10
20
30
40
Total Population
Among Alcoholics
LIFETIME PREVALENCE (wtd. %)

FIGURE 3

The Symptomatic and Prognostic Implications of Psychiatric Diagnoses in Treated Substance Abusers

T. Kosten

INTRODUCTION

Despite theoretical propositions and empirical findings suggesting that psychopathology is common in opiate addicts, treatment has traditionally focused on social rehabilitation and control of substance abuse, while placing little emphasis on treatment of psychiatric disorders. This lack of focus on psychopathology has been addressed in more recent studies, and the importance of systematic, categorical psychiatric diagnosis has been underlined by recent developments in biological psychiatry. Advances in psychiatric genetics and psychopharmacology have tenatively suggested specific treatments and biological abnormalities associated with the major psychiatric disorders. Particularly for affective disorders pharmacotherapy has been demonstrated to have specific efficacy with lithium treatment for bipolar disorders and with tricyclics and other antidepressants for unipolar depressions. Detecting these treatable conditions within clinical populations is clearly important, and this detection might rely on an initial screening using symptom scales followed by the more resource intensive application of categorical diagnoses to selected patients.

This paper will focus on depression, because it is the most prevalent disorder among addicts, but the paper will also address antisocial personality disorder and more general psychopathology. The treatment implications of three questions will be addressed. First, what are the rates of major psychiatric disorders in substance abusers, particularly alcoholics, opiate addicts and cocaine abusers? Second, what is the course of depression among substance abusers? Third, what are the prognostic implications of psychiatric comorbidity? To address this question substance abusers with and without comorbid diagnoses such as depression will be compared using several follow-up studies in which substance abusers were first diagnostically assessed on entrance to treatment and then reevaluated six months, one year and 2.5 years later. While treatment outcome in substance abusers has most commonly focused on two relatively limited definitions of success - retention in treatment or remaining drug-free, this paper will use a multi-dimensional approach. This approach includes medical, psychological, employment, legal, family and social problems as important and relatively independent outcome measures that may not necessarily improve with sustained abstinence or program participation or which may improve without abstinence or program retention.

WHAT ARE THE RATES OF PSYCHIATRIC DISORDERS IN SUBSTANCE ABUSERS?

The most common disorders among substance abusers are depression, antisocial personality disorder and concurrent abuse of a secondary drug (e.g. alcohol for opiate addicts). Among alcoholics secondary drug abuse has the highest rate (43%), followed by antisocial personality (41%) and major depression (38%). Phobias are also common (27%). Having any lifetime disorder is quite common at 77%. Among opiate addicts major depression is most common (54%), followed by alcoholism (35%) and antisocial personality (26.5%). Phobias are less common (16%), and having any lifetime disorder (87%) is even higher than among alcoholics. Among cocaine abusers major and minor depressions are the most common disorders (about 30%), but the next most common disorder is bipolar disorder (10-20%). Concurrent alcoholism is commonly encountered clinically, but antisocial personality has been relatively uncommon in studies over the last few years. Thus, depressive disorders, which are amenable to a number of treatment approaches appear to be quite common among substance abusers.

WHAT IS THE COURSE OF DEPRESSION AMONG SUBSTANCE ABUSERS?

Recent drug use may have an effect on the presentation of a substance abuser in a current depressive episode. Opiate abusers with more severe drug abuse are more likely to be depressed. Opiate addicts first entering treatment are more likely to be depressed than those on methadone maintenance (29% vs 20%). At higher methadone doses depression is more likely, particularly comparing under 30 mg to over 60 mg daily. For these two patient groups the rates of depression were 8% and 39%, respectively. Concurrent alcoholism also increases the risk of depression in opiate addicts from 22% to 34%.

Major depression appears to clear up during long term follow-up. During a 2.5 year follow-up of opiate addicts, only 5.4% were depressed at both the initial and follow-up evaluations, and 20.5% had recovered. New cases of depression had occurred in 7% of the patients, but 67% were not depressed at either evaluation. About half of these 67% had been depressed some time in the past, while not being in a current episode of depression at either evaluation. Thus, as indicated earlier the lifetime rate of depression was 54% overall among these patients.

WHAT ARE THE PROGNOSTIC IMPLICATIONS OF PSYCHIATRIC COMORBIDITY?

In examining outcome this paper takes a multidimensional approach rather than focusing on the more traditional unidimensional outcomes of drug abstinence and treatment retention. Among the various supports for a multidimensional approach, the most graphic indication of the relative independence of outcome areas is the intercorrelation among an array of outcomes. In a six month follow-up of opiate addicts only five of 21 possible intercorrelations among 7 outcomes were significant, and the largest amount of shared variance was only 25%. The outcomes included weeks of treatment, weeks of illicit opiate use, weeks of alcohol use, number of arrests, weeks worked, social adjustment and psychological symptoms. The intercorrelated areas were between social and psychological adjustment, and the drug abuse and treatment retention outcomes were unrelated to these psychosocial outcomes. Similarly, at a 2.5 year follow-up, the shared variance among outcomes was generally quite low with the most significant intercorrelations between psychological and social problems which were also associated with medical problems. Drug abuse was only associated with legal problems and no other outcome. Thus, a multidimensional approach to outcome seems justified.

The prognostic significance of comorbid psychiatric disorders appears to vary for the different disorders. Among opiate addicts, major depression at intake predicted more severe occupational, drug use and psychological problems at a 6 month follow-up, while antisocial personality disorder predicted more severe legal problems. Diagnosis at entrance to treatment also predicted more severe problems at a 2.5 year follow-up. For major depression, current functioning and psychosocial adjustment during the 2.5 years were worse than for non-depressed patients. For anxiety disorders, current functioning was also worse than for non-anxious patients. For antisocial personality, psychosocial adjustment was worse, but interestingly legal problems were not worse than for non-antisocial patients. For opiate abusers with concurrent alcoholism, no differences in outcome were found compared to the non-alcoholics. No differences were found in substance use and medical disability outcomes among abusers with psychiatric comorbidity. Using the psychological problems scale of the Addiction Severity Index as a measure, general psychopathology can also be shown to predict worse long term outcome among opiate addicts. On all five outcome measures from our 2.5 year follow-up - current functioning, psychosocial adjustment, substance use, legal problems and medical disability - those patients with medium to high severity of psychological problems at intake had worse outcomes at follow-up than the low severity patients. The highest severity patients did particularly poorly in current functioning, psychosocial adjustment and substance use suggesting some relationship between continued drug abuse and impairment in other areas, a unidimensional view of outcome.

Depressive disorders may also predict subsequent abstinence or relapse to drug abuse. In our 2.5 year follow-up, we examined recent life events as precipitants of relapse to drug abuse in opiate addicts who either dropped out or remained in treatment. We found that former addicts who remained in treatment were more likely to remain abstinent (43% vs 58%), while those who were depressed at intake or who reported recent life events within 6 months of the follow-up evaluation were less likely to be abstinent (39% vs 50% for depression) (35% vs 54% for recent life events). Exit events, such as a divorce or death of a family member, were particularly associated with less abstinence (39% vs 64%). These three factors - treatment, recent life events and depression - also had an additive effect on abstinence or relapse. Patients who had no exit events, were not depressed at intake and remained in treatment had the highest rate of abstinence (75%), while patients who experienced exit events, were depressed at intake and had dropped out of treatment had three times lower rate of abstinence (24%). For every combination of treatment status and exit events (except the no treatment patients without exits), the depressed were less likely than the non-depressed patients to be abstinent. Thus, the long term prognostic significance of depressive disorders for relapse to drug abuse may be related to a patient's ability to cope with life stresses, particularly losses.

Among alcoholics, psychopathology was associated with more severe psychosocial problems and alcohol dependence. Patients with primary major depressive disorders had more impaired control, psychological problems and alcohol dependence than those without these disorders at a 6 month follow-up. Patients with antisocial personality disorder also had more impaired control and social problems than those without this disorder. Alcoholic patients with concurrent drug abuse had more impaired control, social and psychological problems than "pure" alcoholics. At a one year follow-up, similar results were obtained for depressed men who more frequently had pathological patterns of alcohol use, social impairment, withdrawal symptoms from alcohol, physical conditions due to alcohol, more intense drinking, and more psychopathology on the MMPI than those without depression. However, in most of these areas major depression showed the opposite prognostic pattern for women alcoholics. Only on the MMPI measure of psychopathology did depressed men and women both have higher levels than non-depressives; otherwise, at follow-up depression in women appeared to be associated with fewer problems in these alcohol related areas. Similarly, the men with antisocial personality had more alcohol related problems and MMPI psychopathology than the non-antisocial men, but the antisocial women appeared to have fewer problems than the non-antisocial women in every area except MMPI assessed psychopathology. These findings for women alcoholics are quite different from the majority of studies, but most research samples have been dominated by the more common male substance abusers. Using a more global assessment of psychopathology for one year outcome, differences are still evident between men and women alcoholics, but in general the direction of the associations are more consistent with other studies. For women in particular, more severe psychiatric severity at intake was correlated with more severe alcohol and psychosocial problems including pathological patten of use (r=0.27), social impairment (r=0.26), withdrawal symptoms (r=0.25), poorer social adjustment (r=0.30), total drinking days (r=0.22), intensity of drinking (r=0.32), MMPI psychopathology (r=0.64) and the Cornell Medical Index (r=0.34).

WHAT ARE THE IMPLICATIONS FOR TREATMENT PROGRAMS?

Since comorbid psychiatric disorders appear to have prognostic significance, it is important to detect these disorders in substance abusers and provide appropriate treatment. A two step process may be useful for this purpose. Step 1 is to screen patients for these disorders, since the resources needed for a full diagnostic evaluation are not available for every patient in most community programs. A reasonable screening battery might include the Addiction Severity Index and Beck Depression Inventory and for programs that do not specialize in alcohol treatment, the Michigan Alcoholism Screening Test (MAST). These tests are very useful, because they are either self reports or can be administered by non-professional staff with relatively little training. Step 2 is to establish diagnoses in patients who are identified as being psycholcgically impaired on the screening instruments. These instruments such as the Diagnostic Interview Schedule (DIS), Schedule for Affective Disorders and Schizophrenia (SADS), and Structured Clinical Interview for DSM-III-R are much more labor intensive and usually require extensively trained personnel or professionals for proper administration. Their importance lies in their identifying specific disorders for which pharmacological and psychotherapeutic interventions have been developed. Review of these interventions is beyond the purpose of this paper, but particularly for depressive disorders a number of interventions now have demonstrated efficacy among substance abusers and should be applied in order to improve treatment outcome.

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Behavioral Concomitants of Ethanol and Drug Reinforcement

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Both ethyl alcohol and marihuana are two of the oldest used and abused substances, yet the neural mechanisms underlying intoxication and resultant reinforcing properties of these drugs are unknown. In fact, the abuse of ethanol is quite paradoxical since relaxation and pleasurable effects after moderate consumption rapidly changes to dysphoria and anxiety as drinking progresses. The chronic ethanol abuser is also plagued by bouts with anxiety, depression (cf. Mendelson, 1964; Mello, 1972; Nathan *et al.*, 1970) as well as a deterioration of vital organ function. Similarly, the first few experiences with marihuana may evoke anxiety which is perhaps due to delta-9-tetrahydrocannibinol (Δ^9-THC)-induced tachycardia.

The present study measured a number of behavioral, electrophysiological and endocrine events immediately after exposure to various doses of ethanol or marihuana in an attempt to determine which measures co-vary with the pleasurable components of the drug effect.

METHODS

Subjects: Adult male and female volunteers between the ages of 21 and 35 years were recruited via newspaper advertisements and provided informed consent for participation in this study. Subjects were examined by a specialist in internal medicine and only those with normal physical examinations, medical and mental health histories and blood hemogram and chemistry studies were admitted to the study. Urine specimens were screened for psychoactive drugs and all results were negative. No subject had a history of drug or ethanol abuse, but all subjects described themselves as social drinkers who, on the average, consumed the equivalent of 3 beers per week.

Experimental Design and Setting: Effects of two doses of ethanol or marihuana were compared with placebo in a group design (n=6 per group) under randomized double-blind conditions. The studies were conducted within the context of a multidisciplinary program designed to assess EEG, physiologic, endocrine and behavioral responses following acute ethanol or marihuana administration. Results obtained using the instrumental device were previously validated and compared with verbal reports of subjective mood states (Lukas *et al.*, 1986a,c,d). Subjects were told that they could receive either "placebo, a low dose of alcohol (about 1 shot) or a high dose of alcohol (about 3 shots)." Six subjects received placebo (350 ml of concentrated grapefruit juice plus 3 ml of ethanol "primer" as described below), six subjects received 0.347 g/kg of ethanol (1.1 ml/kg of 80 proof vodka in 350 ml of fruit juice) and six subjects received 0.695 g/kg of ethanol (2.2 ml/kg of 80 proof vodka in 350 ml of fruit juice). Similar instructions were given to subjects in the marihuana study. All cigarettes weighed about 950 mg. Placebo, low-dose, and high-dose marihuana cigarettes contained 0.004%, 1.26% and 2.53% Δ^9-THC, respectively.

All studies were conducted in an electrically shielded, sound- and light-attenuated double-walled chamber (IAC, Bronx, NY). The chamber was equipped with a wired intercom and a one-way glass window for maintaining auditory and visual contact with the subjects. Subjects sat in a comfortable reclining chair and were instructed to relax and keep their eyes closed, but to remain awake.

Electrophysiologic Recording Procedures: Electroencephalographic and physiologic activity were recorded on a Model 78D polygraph (Grass Instrument Co., Quincy, MA). Scalp EEG electrodes (C3, C4, P3 and P4) were referenced to linked earlobes and were applied using the International 10-20 System (Jasper, 1958). Temporalis muscle electrodes were used to record muscle tension, a Grass TC-1 thermocouple electrode was attached to the subject's nostril via a plastic clip to record respiratory rate and a Grass PTTL-F photoelectric transducer was attached to a finger to record the subject's pulse. The EEG activity was also recorded on FM magnetic tape for off-line power spectral analysis using a Pathfinder Signal Averaging Microprocessor (Nicolet Instrument Co., Madison, WI). A digital time-code was also recorded on one channel of the magnetic tape to facilitate identification of specific epochs of EEG activity during off-line computerized power spectral analysis.

Blood Sampling Procedures: Blood samples for analysis of integrative plasma ethanol concentrations were withdrawn continuously during the entire study. A 183 cm Kowarski-Cormed Thromboresistant Blood Withdrawal Butterfly Needle and Tubing Set (Cormed Inc., Medina, NY) was used for blood sampling. The extra long length was necessary so that blood could be drawn from outside the chamber without disturbing the subject. The tubing was attached to a 10 ml syringe mounted on a withdrawal syringe pump (Harvard Apparatus, Cambridge, MA) and adjusted to withdraw blood at a rate of 1 ml/min; syringes were changed every 5 min. Plasma samples were immediately prepared and frozen for subsequent ethanol concentration analysis by the spectrophotometric method of Leric *et al.* (1970); Δ^9-THC levels were measured using a radioimmunoassay kit.

Behavioral Measures of Subjective Mood States: A custom-designed manipulandum resembling a joystick device was placed next to the subject's left hand (Lukas *et al.* 1986a,c). Movement of the joystick produced a corresponding deflection of a polygraph pen while pushing the button located on the top of the joystick activated an event pen on the polygraph. A second button located below the first activated a second event pen for indicating dysphoria. The joystick could be moved either forward, backward, or to one side and was spring-loaded so that it returned to the center position once it was released. Less than 1 Newton of force was required to move the joystick to one of the three different positions.

All subjects were read the following instructions regarding the definition of ethanol-related effects: "You may use any of the following terms to describe alcohol effects or intoxication: giddy, light-headed, buzz-on, high, drunk, or euphoria." Subjects were then instructed to operate the joystick as follows:

Forward--"when you feel intoxicated or under the influence of alcohol"; Side--"when you feel that these effects are getting stronger"; Backward--"when the alcohol effects disappear"; Button--"when you experience a feeling of intense well-being or euphoria." Self-reports of intoxication were obtained from the subjects with both an instrumental response and a questionnaire. A previous study comparing instrumental and verbal responses reported that dose-related ethanol-induced changes in mood states were detected using an instrumental response but not with the self-rating questionnaire (Lukas *et al.*, 1986a). Consequently, only data obtained with the instrumental device are presented here.

Ethanol Administration: Placebo and drug solutions were placed in an inverted IV bottle and attached to a Masterflex peristaltic pump (Cole Parmer Co., Chicago, IL). Tubing from the pump entered through the wall of the experimental chamber. A disposable mouthpiece was attached to the end of the tubing and was supported by a flexible metal arm located near the subject's mouth. For all three treatments (two doses of ethanol and placebo) a 10 ml reservoir located between the pump and the subject's mouthpiece was filled with 3 ml of vodka (i.e., "primer") and 7 ml of juice to provide a strong initial taste of ethanol. This procedure for disguising the solution's taste has proven useful as an effective ethanol placebo control. The small amount of ethanol in the placebo solution does not produce any measurable plasma ethanol levels (Mendelson *et al.*, 1984; Lukas *et al.*, 1986a,c).

Marihuana Administration: Placebo and marihuana cigarettes were affixed to a custom-designed trap bottle located outside of the experimental chamber. A flexible plastic tube was attached to the vented side of the bottle and was passed through the chamber wall A standard cigarette-holder mouthpiece was attached to the distal end of the tubing and was supported by a flexible metal arm so that the subject was free to leave one hand on the joystick device while smoking. The smoke was cooled and filtered by routing it through cold water contained within the trap bottle. In addition, a vacuum transducer was attached to the plastic tubing which was connected to an event pen to provide a direct record of individual puffs on the polygraph. Specific instructions for smoking the cigarette were recorded on magnetic tape and played for the subject as follows: "inhale" for 3 sec, "hold" your breath for 5 sec and then "exhale". This sequence was repeated every 30 sec until 10 mm of the cigarette remained unsmoked. All subjects finished smoking within 10 - 13 min.

Procedure: Baseline EEG, physiologic and behavioral data were recorded during the first 30 min of the study. During this control period subjects were asked to move the joystick and press the button while the EEG was observed for movement-related artifacts. The EEG during these responses was analyzed as described below. After the 30 min baseline period, the subject was instructed to place either the drinking or smoking tube mouthpiece comfortably into his mouth and the peristaltic pump was activated to deliver 350 ml of solution over a 15 min time period (ethanol) or the cigarette was lit. Continuous measures of EEG, physiologic and behavioral responses and blood samples were obtained for the next 2 hours.

Data Analysis: Pulse rate, muscle tension and respiratory rate were measured each min during the first 60 min after drinking or smoking and at 5-min intervals for the subsequent 60 min. Movements of the joystick indicating drug detection and episodes of euphoria were directly recorded on the polygraph. Discrete, 2 min artifact-free epochs of EEG activity were selected every 15 min for power spectral analysis using Frequency Analysis Software developed by Nicolet Instrument Co. The process digitized the analog waves at a rate of 256 Hz, performed a Fast Fourier Transformation on the sample and then generated a compressed spectral array representing EEG power as a function of frequency. The corresponding power ($\mu V^2/Hz$) and peak frequency (Hz) in the 0.25-4 Hz, 4-8 Hz, 8-13 Hz and 13-30 Hz bands were quantified and printed for 20 sec epoches of EEG activity.

In addition, topographic mapping of brain electrical activity was conducted in six female subjects after placebo and 0.7 g/kg ethanol. EEG activity from 25 scalp electrodes were processed using Brain Electrical Activity Mapping (BEAM™) technology (Duffy *et al.*, 1979). Details of the recording, artifact rejections, and display procedures are available from the authors.

Statistical analysis was performed on an Apple IIe microcomputer using software developed by Human Systems Dynamics (Northridge, CA). Analysis of variance with repeated measures followed by Dunnett's significant difference text, linear regression, and correlation and covariance tests were utilized as necessary. Tests for parallelism were conducted using the method of Tallarida and Murray (1981).

RESULTS

Subjective reports of intoxication, as indicated by joystick responding, were evident after both ethanol and marihuana administration. Subjects clearly discriminated between simple detection of ethanol or marihuana effects (joystick position) and a qualitative measure of intense pleasure or euphoria (button pressing). Subjects typically detected drug effects 10-20 minutes after initiation of drinking or smoking and reported a decrease in response 60-90 minutes later. An unexpected finding was that subjective reports of euphoria were paroxysmal in nature, averaging 2-8 minutes in duration and occurred about 3-5 times during the first 40 minutes after drug administration. Plasma ACTH levels peaked at this time. Subjects also provided verbal confirmation of the fleeting nature of the response during post-session debriefing. Responding after placebo ethanol was characterized by a longer delay to detect, shorter overall duration, and no euphoria. All subjects were able to discriminate placebo marihuana from active.

Quantitative measures of brain electrical activity using power spectral analysis revealed that EEG alpha activity increased abruptly during these brief reports of euphoria. This was observed after both ethanol and marihuana, but was absent in subjects who received placebo. Furthermore, these alterations occurred as plasma ethanol and THC levels were rapidly rising. Concomitant measures of P300 auditory evoked potentials revealed that disruption of selective attention did not parallel reports of euphoria, but instead reflected the intensity and duration of intoxication.

Topographic mapping of brain electrical activity during ethanol-induced intoxication revealed that increases in EEG alpha power were widely distributed over the scalp and encompassed parietal and frontal scalp electrodes. These areas are usually devoid of prominent alpha activity during control recordings. Furthermore, this distribution of brain electrical activity was not observed in any subject after placebo ethanol administration. However, one female subject who reported a positive family history of alcoholism, also failed to exhibit either behavioral or electrophysiological signs of intoxication in spite of relatively high plasma ethanol levels.

DISCUSSION

The present study demonstrated that acute administration of both ethanol and marihuana results in pronounced behavioral effects that were temporally related to alterations in brain electrical activity. Such a relationship might reflect the nature of reinforcement processes. Abrupt increases in EEG alpha activity are associated with a pleasurable, free-floating and extremely relaxed state (Lindsley, 1952; Brown, 1970; Matejcek, 1982) similar to that induced by transcendental meditation (Wallace, 1970). The finding that both ethanol and marihuana produce increased alpha EEG activity suggests that this neurophysiologic index may be intimately associated with the processes that control feelings of well-being.

The results presented here suggest that pleasurable drug-related experiences are reported during a time of rapid transition between a sober and intoxicated behavioral state. For example, the paroxysmal episodes of subjective reports of intense pleasure or euphoria after either ethanol or marihuana suggest that under the present laboratory conditions, the reinforcing mechanism is intense, but fleeting in nature. Furthermore, the temporal correlation among EEG, behavioral and endocrine effects of ethanol and marihuana suggest that these processes may be related. Such rapid intense sensations of intoxication are comparable to the heroin rush and the cocaine high that persist for seconds or minutes and then rapidly disappear.

Throughout this report a distinction has been made between detection or identification of drug effect, and intense pleasure or euphoria. Without a continuous probe of behavioral states, such rapid transitions cannot be detected. During debriefing after a study, subjects who received active drug are universally surprised that the pleasurable effects of the drug were so brief and episodic in nature. The precise mechanism of these transient episodes of drug-induced euphoria remain an enigma. The precise mechanism underlying these transient behavioral and EEG changes remain unknown. However, the concomitant pulse of ACTH during ethanol-induced euphoria suggests that the mechanism may very well involve the hypothalamic-pituitary axis.

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Opiate-Ethanol Interactions: Implications for the Biological Basis and Treatment of Combined Addictive Diseases

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Co-existence of narcotic abuse and ethanol abuse, and combined narcotic and alcoholic addictive disease, are common [8,11,12,14,27,28]. Both require specialized clinical management. Relations between these two clinical problems may or may not bear any direct relationship to the undefined mechanisms underlying each separate addictive disease entity, and to the production and maintenance of the tolerant and dependent state. Drug interactions may occur between opiates and ethanol which alter pharmacological properties and the various effects caused by each, and in turn may contribute to the addictive diseases. Types of opiate-ethanol interactions include two theoretical possibilities: 1) dispositional interactions, which include changes in absorption, distribution, metabolism and excretion, resulting in alterations in pharmacokinetics; and 2) pharmacodynamic interactions, either of a general non-specific type, or at a specific site of drug actions such as a receptor site. The results of opiate-ethanol interactions could include additive, synergistic, diminished or antagonistic effects of pharmacological, physiological, pathological or toxicological types [5,6,8,10-15,16,18-21,24, 29-31].

In the United States it is estimated that there are now at least 2 million persons who have used heroin at some time, and at least 500,000 long-term heroin addicts. It is estimated that there are 164 million persons who have ever used ethanol with 11,500,000 problem drinkers, and 5,750,000 alcoholics. In the 1985 DAWN survey report, alcohol in combination with other drugs was the leading cause (20%) mentioned by emergency rooms, of acute medical problems related to drug abuse [28]. Heroin was the second most commonly mentioned drug in acute medical emergency problems (13.9%). Over 40% of the heroin cases were medical emergencies where alcohol was also present. Alcohol in combination with other drugs accounted for 31.6% of medical examiner reports of deaths related to or caused by drug abuse; heroin, often in combination with alcohol, accounted for 27.4% of the deaths. Thus it is evident that alcohol in combination with heroin, is a very common cause of major medical problems leading to medical emergencies severe enough to require emergency room visits, in many cases resulting in death. With respect to possible drug interactions between the heroin and ethanol, it is of interest to consider what are the patterns of the combined use. Heroin used concomitantly with ethanol apparently results in the desired effect of enhancement of the heroin "high" or euphoria, whereas, heroin use followed several hours later by the use of alcohol apparently has the desired side effect of self medication of narcotic withdrawal symptoms. These patterns of drug abuse may provide some insights into the actual drug interactions of a pharmacokinetic and pharmacodynamic type which may occur. Similar patterns of alcohol abuse may occur in some patients during chronic methadone maintenance treatment for narcotic addiction.

Since drug interactions may result in altered toxicity, physiological or pharmacological effects, it is necessary to consider the toxicities and effects of each drug when used alone. Alcohol is an intrinsic hepatotoxin, neurotoxin and cardiotoxin, and is also directly toxic to the gastrointestinal tract and pancreas. Of especial importance with regard to drug interactions, ethanol is a powerful enhancer of specific hepatic microsomal P450 dependent drug metabolizing enzyme activities. Therefore, when alcohol has been used on a chronic basis, the hepatic drug metabolizing enzyme capacity is enhanced. Conversely, when alcohol is acutely administered in high doses, either to a naive subject or a chronic user of alcohol, alcohol may inhibit drug metabolism by certain hepatic P450 dependent microsomal enzymes. This is related to the fact that when present in high doses, 25-30% of ethanol is itself metabolized by a hepatic P450 dependent microsomal enzyme system in addition to its major pathway by cytosolic alcohol dehydrogenase. Therefore, alcohol has two very distinct and opposite effects on drug metabolism: inhibition of drug metabolism when present in large amounts, and enhancement of oxidative drug metabolism when alcohol has been used on a chronic basis and is then withdrawn. This dual effect by alcohol on metabolism of another drug has been documented for many different drugs. Alcohol is also a modifier of several physiological functions, with resulting abnormalities of endocrine, reproductive, gastrointestinal, and immune functions.

Heroin, on the other hand, is not intrinsically toxic to any organ system. There is no evidence that heroin, or any other opiate such as morphine or methadone, has any effect on hepatic P450 drug metabolizing enzyme activities in humans, although such effects may occur in other species. Heroin, and other narcotics, when given in very large doses may result in respiratory depression with resultant hypoxia which can cause toxic changes in all tissues and organs. Frequently in animal model studies, such changes are incorrectly ascribed to direct toxic effects of the narcotic, rather that correctly ascribed to the results of severe hypoxia. Like ethanol, heroin may be a potent modifier of physiological functions.

When research on the possible long-term pharmacological treatment of narcotic addiction was initiated by Dole and colleagues in 1964, a long-acting orally effective opioid, methadone, was chosen for use [3]. It was shown that steady daily dose administration of methadone provided several needed effects, including prevention of abstinence symptoms over a dosing interval of 24 hours, prevention of drug hunger and drug seeking behavior, and normalization of overall status. In other attempts to treat narcotic addiction pharmacologically, when short-acting narcotics such as heroin or morphine were used, it had been repeatedly demonstrated that repeated parenteral doses of narcotic, usually in increasing doses, were required to prevent narcotic withdrawal and drug hunger. Such frequent dosing with a short-acting narcotic, leads to high peak plasma levels followed by rapid decline in plasma levels and results in intermittent periods of narcotic effects followed by a very short period of relatively normal functional status and then by a period of narcotic withdrawal.

Subsequently, when pharmacokinetic studies could be carried out using both standard techniques, and stable isotope labeled tracer techniques, it was shown that methadone is essentially completely systemically bioavailable after oral administration, with an apparent plasma terminal half-life of 16-28 hours for the dl racemic compound used in therapeutics, and up to 48 hours for the active l-enantiomer [4,9,21,22,27]. This was to be sharply contrasted with the pharmacokinetics of heroin which has a plasma terminal half-life of approximately 4-6 hours, and very limited systemic bioavailability after oral administration (less than 30%), thus requiring parenteral administration. Methadone is metabolized primarily by the hepatic microsomal P-450 drug metabolizing enzymes, undergoing oxidative metabolism with N-demethylation followed by cyclization as the major route of biotransformation in humans [9,19]. Conversely, heroin is metabolized primarily by deacetylation followed by glucuronidation of the resultant morphine in humans.

In clinical pharmacodynamic and pharmacokinetic studies, when methadone is given in an adequate dose orally once a day, a steady state plasma level of methadone is sustained over a 24 hour dosing interval. Peak plasma levels of methadone occur 2-4 hours after the oral dosing, and represent barely a doubling of the nadir of steady state background levels. Thus, in addition to clinical observations of normalization of behavior and general physical functioning, it was demonstrated pharmacologically that a steady state of plasma levels of methadone is achieved, and presumably a steady state of opioid is available at specific opiate receptor sites. It is this steady state of plasma and receptor levels of this long-acting opioid that probably allows normalization of many physiological functions which are disrupted by acute use of any opioid and remain disrupted during chronic use (such as in cycles of addiction) of short-acting narcotics, of which heroin is the most commonly abused drug.

To date, over 150,000 former heroin addicts have been treated with methadone maintenance treatment, and approximately 100,000 are currently in treatment. The voluntary retention in methadone maintenance treatment for more than 2 years ranges from 55-80%, with retention higher in clinics well structured to provide general medical and behavioral services, along with counseling and rehabilitation services. As shown by many studies, less than 10% of long-term methadone maintained patients continue to show illicit narcotic abuse. However, several studies have now documented the early observations that relapse rates after cessation of methadone maintenance treatment are very high. From 70-80% of former well rehabilitated methadone maintained patients will ultimately return to narcotic use, with increased prevalence of ethanol abuse often preceding the return to illicit narcotics. These data suggest that for a large proportion of methadone maintained patients, chronic long-term treatment is essential.

The actions of methadone treatment include three important separate effects: 1) prevention of withdrawal symptoms over a 24 hour dosing interval; 2) prevention of "drug-hunger" (whatever ultimately turns out to be the physiological and/or behavioral basis of this well-documented entity); and 3) blockade of the euphoric effects of any illicitly self-administered narcotics, by the well studied phenomena of opiate-cross-tolerance [3]. The mechanism of action of methadone in maintenance treatment is probably related to the fact that the long-acting opioid provides steady levels of opioid at receptor sites, thus allowing normalization of functions disrupted by intermittent receptor occupancy.

It has become quite clear from both early and more recent studies that alcohol abuse, a very common problem in street heroin addicts, also remains a problem of patients in methadone maintenance treatment [1,5,6,8,10-15,20,21,29-31]. Between 20% and 50% of heroin addicts are abusers of alcohol or are alcoholics by definition; several studies have also shown that between 25-50% of patients in methadone maintenance treatment continue to use alcohol to excess or are alcoholics. Contrary to earlier predictions, it has been shown that alcohol abusing, or alcoholic methadone maintained patients do benefit from methadone maintenance treatment with respect to cessation of heroin addiction but continue to have the second alcohol addictive disease process [1,5]. Therefore, in the current setting of an increasing AIDS epidemic in which parenteral drug abusers are the second highest risk group, it is important to address the second addictive disease of alcoholism in methadone maintained patients, rather than discharging alcohol abusing or alcoholic methadone maintained patients from methadone treatment. However, there are many indications, both from laboratory and clinical studies, that concomitant alcohol abuse, through dispositional interactions with the opioid methadone, may disrupt the steady state which otherwise may be achieved during chronic methadone maintenance treatment. By altering the disposition and pharmacokinetics of methadone, normalization of various functions of potential importance, including neuroendocrine functions, may be prevented or impaired.

Along with other workers, we have carried out studies in rat models to determine what may be the interactions between methadone and ethanol. In one such study, rats were treated with methadone (25mg per kg per day po), or water daily by gavage for two weeks. Rats were then treated concomitantly with methadone and either ethanol (2g per kg per day po), or isocaloric sucrose for two weeks. On the day following the last dose of ethanol administration, rats were sacrificed at sequential time points following the last oral dose of methadone and plasma levels of methadone were determined by gas chromatographic methods developed in our laboratory. In these studies it was shown that plasma levels of methadone at one and two hours after the last oral dose of methadone was administered, and 24 hours after the last dose of ethanol, were significantly lower in animals which had been treated with ethanol as contrasted with the animals which had received isocaloric sucrose. These studies therefore demonstrated the capacity of chronic ethanol treatment to accelerate or enhance methadone metabolism [10,12,15].

Studies have also been performed in an animal to determine whether or not the impairment of methadone metabolism, which is postulated to occur when high levels of ethanol are administered concomitantly with opioid, are due to acute effects of ethanol on hepatic uptake and distribution. A series of studies were carried out using an isolated perfused rabbit liver preparation [24]. We had earlier shown that morphine, which has very limited systemic bioavailablity after oral administration, was extracted by the liver to a much lesser extent (a paradoxical finding) than both methadone and l-alpha-acetylmethadol, two opioids which are long-acting in man and which have very high systemic bioavailability after oral administration. This paradox was explained by demonstrating that, whereas morphine undergoes rapid biotransformation (N-demethylation in most animals and glucuronidation in humans) in its "first pass" through the liver, substantial amounts of unchanged methadone and LAAM were initially nonspecifically bound to hepatic plasma membrane fractions and subsequently slowly released. Therefore, both in the isolated perfused rabbit liver preparation, and in whole animal (rodent) preparations, as well as in compartmental pharmacokinetic analyses of methadone disposition in human subjects after the administration of stable isotope labled enantiomers of methadone, it has been shown that the liver is an important reservoir for the storage and subsequent release in unchanged form of these long-acting opioids [4,22,27,30,31]. In studies carried out to determine the effects of ethanol preperfusion on the acute hepatic uptake and distribution of narcotics in the isolated perfused rabbit liver, isolated rabbit livers were perfused with rabbit blood, both with and without ethanol added to maintain ethanol levels around 200mg/dl. After a 75 minute of pre-perfusion, a pulse injection of ^{14}C labeled narcotic (1.5mg) was made into the portal vein cannula followed by a two minute, open, non-recirculating perfusion. At the end of the two minute perfusion, the entire liver was homogenized, sub-cellular fractionation performed, aliquots of whole liver homogenate and sub-cellular fractions oxidized and radioactivity content determined. In these studies, it was shown that alcohol pre-perfusion and co-perfusion had no observable effects on the hepatic uptake of either of the short-acting narcotics studied, morphine and meperidine, nor on the hepatic uptake of the long-acting narcotics, methadone and l-alpha-acetylmethadol [24]. Similarly ethanol pre-perfusion and co-perfusion had no effects on the sub-cellular distribution of these opioids in the liver in this acute perfusion system [24]. Therefore, it would seem that when ethanol is present in large amounts, it acutely inhibits narcotic metabolism; this effect is probably exerted at the level of the hepatic microsomal P-450 drug metabolizing enzymes.

Studies were then carried out to determine what may be the extent and nature of interactions between this long-acting opioid, methadone, and ethanol in human subjects in methadone maintenance treatment. First, a study was carried out in chronic methadone maintained patients who had no history of alcohol abuse, polydrug abuse, or significant liver disease [2]. Five subjects were studied both while receiving methadone alone in steady-state treatment doses (30-100mg/day orally), and then on a special study day when ethanol (90ml of a 50% solution) was co-administered with a morning daily dose of methadone. In this study the ethanol dose administered, equivalent to 45 ml of ethanol, did not cause any significant alteration in methadone disposition [2].

A study is currently in progress to determine the interactions between ethanol and methadone in alcoholic methadone maintained patients [23]. In this study methadone maintained patients with identified alcoholism and who have been voluntarily admitted for detoxification treatment for alcoholism, and to participate in this pharmacokinetic study, are admitted to a clinical care unit where research studies may also be performed [23]. The patients are stabilized for 2-3 days on the minimum doses of ethanol required to prevent alcohol withdrawal symptoms. On the third or fourth day of hospitilization, a study is performed in which the stabilizing dose of ethanol is administered at the beginning of the day along with the daily oral dose of methadone used in maintenance treatment. Preliminary data analyses suggest that in those "subjects receiving an initial ethanol dose equal to or greater than 75ml equivalent of ethanol on the study day", the methadone dose adjusted area under the plasma concentration time curve (AUC_{0-24}) is significantly higher during the ethanol study, than when those same subjects are studied two weeks following cessation of ethanol treatment. Therefore, these studies may provide quantitative evidence for the first time which supports the clinical evidence that there is a dispositional interaction between ethanol and methadone in man following acute large doses of ethanol, resulting in elevation in plasma levels of methadone during the time course when ethanol was present in the body [23]. However, these preliminary studies, as well as the animal model studies, may show that following withdrawal of ethanol, plasma levels of methadone may be lower than anticipated for the dose of methadone received.

Chronic liver disease resulting in part from chronic alcohol use may also alter methadone disposition. It has been shown in several studies that from 50-70% of all heroin addicts and methadone maintained patients have laboratory evidence of chronic liver disease [8,20]. A history of clinically evident hepatitis is present in only 20-30% of patients. It has been shown that chronic hepatitis B antigenemia is present in 5-15% of heroin addicts and also patients in chronic methadone maintenance treatment. Other serological markers of hepatitis B infection (including HBsAb and HBcAb), may be found in 50-75% of patients. More recently, there is evidence of an increasing prevalence of hepatitis delta infection with serological markers found in 15-30% of heroin addicts and methadone maintained patients studied. As stated above, a history of chronic heavy usage of ethanol (greater than the equivalent of 4oz of whiskey per day), may be elicited in 20-50% of both heroin addicts and methadone maintained patients. It has been shown that liver disease due both to alcohol abuse and viral liver disease may progress more rapidly than disease due to viral infection alone [1,5,8,20,29].

We have carried out two sets of studies of methadone disposition in patients with alcoholic cirrhosis, at a time when there has been no alcohol ingested for at least 2 weeks prior to study [30,31]. In the first of these studies of compensated alcoholic cirrhotics, all of whom were able to undergo liver biopsy, the one pharmacokinetic index of methadone disposition which was significantly altered was the apparent terminal half-life of methadone, with the control group in this study having a half-life of 18.8 hours for the racemic methadone and the group of subjects with alcohol cirrhosis having a half-life of 35.5 hours [31]. In a second study of patients with more severe alcoholic cirrhosis, some of whom had liver function too impaired to allow liver biopsy, prolongation of apparent terminal half-life of methadone was again documented. However, in this group, the mean plasma level of methadone was found to be significantly lower than the mean plasma level in the control group [30]. In most studies of disposition of other drugs in patients with cirrhosis, in whom biotransformation of the study drug may be compromised because of hepatic dysfunction with resultant prolongation of plasma apparent terminal half-life, accumulation of drug and therefore increased plasma levels are seen. It is postulated that the significantly reduced plasma levels observed in our study of methadone disposition in cirrhotic patients was due to the fact that methadone requires an intact liver for storage and subsequent release. In the setting of severe cirrhosis, with decreased functional hepatic mass, it is probable that the storage capacity for methadone was markedly reduced and that therefore methadone was more extensively, though more slowly, biotransformed in its first passage through the liver, thus resulting in lowered plasma levels over a 24 hour dosing interval. Other studies were carried out to examine the urinary and fecal excretion of methadone and its major metabolites in maintenance patients with and without severe liver disease [18,19]. In these studies it was shown that in a setting of severe liver disease, the urinary excretion of methadone and its metabolites is significantly decreased, reflecting the decreased total plasma load of methadone, whereas, the fecal excretion of methadone and its metabolites is increased, reflecting a probable increased "first pass" effect with biotransformation and hepatobiliary secretion to feces of methadone, rather than storage and subsequent release of methadone in unchanged form into the plasma.

Several workers have recently shown that ethanol may interfere with binding of the specific endogenous opioids to their opiate receptors, and may possibly interfere with the binding of opiate drugs to opiate receptors. Any such disruption of normal binding and release from binding of the endogenous opiates from their receptors, or disruption of binding of narcotic drugs could alter functions modulated or affected by endogenous or exogenous opioids such as neuroendocrine function. Acute administration of either a short- or long-acting narcotic and also chronic administration of a short-acting narcotic, will cause inhibition of release of ACTH and beta-endorphin in humans, along with abnormal release and subsequent lowered levels or altered diurnal circadian rhythm of levels of cortisol. During withdrawal from narcotics, there is increased release of ACTH with increased levels of cortisol. Similarly acute bolus administration of a specific opioid antagonist, naloxone and chronic treatment with the antagonist naltrexone has been shown by our group to result in increased release of beta-endorphin and also increased levels of cortisol [7].

We have hypothesized that during acute and chronic use of short-acting narcotics in humans, with 3 or 4 doses administered each day, and with intermittent periods of narcotic effect followed by relative narcotic withdrawal, alterations in neuroendocrine function may occur. Conversely, during chronic use of the long-acting opioid, methadone, normalization of most indices of neuroendocrine function may result because of the long-acting properties of this drug in humans and the steady-state opiate receptor perfusion which may be achieved during treatment. In the controlled environment of the General Clinical Research Center, we have studied patients who have been in long-term chronic methadone maintenance treatment for 2 years or more who have no significant liver disease, no alcohol abuse and no other polydrug abuse [25,26]. In these studies we have shown that plasma levels and the circadian release of levels of ACTH, beta-endorphin, and cortisol are all normal. This is to be sharply contrasted with our findings in patients their first 1 to 2 months of methadone treatment during which time dose of methadone is being ascended and tolerance to other narcotic effects developing. In that setting ACTH and beta-endorphin release is reduced and cortisol levels are altered, along with disruption of the normal circadian rhythmicity. The effects of alcohol on disrupting methadone disposition, with probable initial reduction of methadone metabolism followed by acceleration of metabolism and resultant disruption of the steady-state plasma levels, and presumably receptor levels of drug, may also disrupt neuroendocrine and endocrine function. Although we do not know the relationships of the disruption of endocrine function to addictive disease, with its major feature of drug seeking behavior, there may be some direct or indirect relationship between the two. Normalized behavior is found in chronic methadone maintained patients around the same time that normalization of endocrine and neuroendocrine function is achieved. It is known that the narcotic abstinence symptoms experienced by alcohol abusing methadone maintained patients at the end of their methadone dosing interval may be directly related to the accelerated metabolism of methadone in that setting. It is not known whether or not any of these effects may also be related to induced alterations in neuroendocrine function.

It has been shown by other workers in normal control subjects and in alcoholic patients that acute high levels of alcohol result in release of cortisol. A so-called "pseudo-Cushing's syndrome" has been observed in chronic alcoholics, and has been related to this hypercortisolemia. However, studies in humans have not yet clarified whether or not alcohol causes an increased release of the controlling peptide, ACTH, and also beta-endorphin released from the same peptide precursor, or whether the alcohol effect is directly on the adrenal cortex, with either a normal negative loop which would result in decreased levels of ACTH and also beta-endorphin, or a disrupted loop which could result in no change in ACTH and beta-endorphin levels. In our ongoing studies in alcoholic methadone maintained patients, we have observed that acute alcohol administration at moderate levels or above results in elevation in levels of serum cortisol [23]. Further studies are in progress to determine the effects of alcohol, when used on acute and chronic basis, on the peptide hormones of the hypothalamic-pituitary-adrenal axis which could have implications both for the biological basis of combined addictive diseases and for the treatment of these disorders.

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The Reinforcing Functions of Drugs and Assessment of Abuse Liability

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Among the many perplexing aspects of the substance abuse domain, self administration models appear to present some of the more challenging methodological and conceptual problems. For at least several decades, this field of inquiry has been cultivated assiduously, and the current lively interest in alcohol and drug self-administration is generously reflected in the combined program of this joint research meeting as well as in an expanding literature of multidisciplinary origins (Brady and Lukas, 1984; Goldberg and Stolerman, 1986; Balster and Lukas, 1985; Ator and Griffiths, 1987). It is unfortunately true however, that dedication and industry, even of the most intense sort, do not guarantee authentic scientific achievement. In biology and particularly the social sciences, wide gaps frequently separate experimental operations and interpretive formulations. Progress in developing systematic and coherent conceptualizations that serve to integrate and unify interactive levels of discourse can be painfully slow. At the most basic level, for example, there appear to be no generally acceptable formulations that bring conceptual order to the rapidly expanding frontiers of inquiry and application in the extended domain of substance abuse. It seems clear, nonetheless, that the development of a unifying framework for conceptualizing the commonalities in alcohol and drug abuse must encompass the data base that focuses upon those behavioral interactions characterized in the title of this symposium as self-administration.

Traditional views of this process as exemplified in alcohol and drug abuse, emphasized its reactive features — attention focused heavily upon the antecedent conditions that appear to "drive" or at least provoke substance use and abuse. But almost three decades have passed since the discovery that animals implanted with intravenous catheters would repeatedly self-inject drugs (Clark, Schuster, and Brady, 1961; Weeks, 1962). And there is now convincing evidence of concordance between the range of chemical substances self-administered by animals and those abused by humans (Brady, Griffiths, and Winger, 1975; Johanson and Balster, 1978; Griffiths and Balster, 1979). Moreover, the variables of which such drug self-administration are a function (e.g., dose, response cost, schedule of availability, environmental conditions, past history) have been found to exert their influence in a similar fashion independently of the type of substance maintaining the performance or the species of organism involved (Griffiths, Bigelow, and Henningfield, 1980; Johanson and Schuster, 1981). The recognition of these cross-species and cross-drug generalities has radically changed conceptualizations of substance abuse from a reactive to a more active process, and has encouraged the kind of functional analysis of drug-seeking and drug-taking that has proven productive and useful in other behavioral interactions (Brady, 1981).

This emphasis upon substance abuse commonalities makes contact with an expanding body of research knowledge based upon the analysis of behavior and is advantaged by the strong empirical influence of the experimental laboratory. The most important point of contact between the experimental analysis of behavior and substance abuse is the demonstrated relationship between the pharmacological properties of drugs and their stimulus functions in behavioral interactions. The aspect of this relationship that dominates investigative attention has continued to focus upon the reinforcing functions of drugs and the conditions under which drug seeking and drug taking behaviors are influenced by their pharmacological properties. As a result, experimental procedures for the generation and maintenance of drug self-administration performances have become the hallmark of abuse liability assessment in both animal and human laboratory settings (Brady and Lukas, 1984). The procedures used most commonly to assess the reinforcing functions of unknown pharmacologic agents involve either direct access to the test compound (via instrumental lever pressing, intragastric catheters, or automatic oral drinking systems) without prior drug self-injection experience (Deneau, et. al., 1969; Altshuler, et. al.., 1975; Meisch and Henningfield, 1977), or substitution of the test compound on a drug self-administration baseline previously established with standard reinforcing agents (Brady, et. al., 1975; Brady and Griffiths, 1976). One variant of the latter procedure described by Griffiths, et. al. (1976) for example, has been used to systematically evaluate the reinforcing efficacy and compare a range of doses of some 15 stimulant anorectics and more than 20 sedative anxiolytics substituted after several consecutive days of multiple cocaine injections per day. Access to each dose of each of the test compounds was permitted for at least 12 days, and Figures 1 and 2 illustrate the resulting comparative analysis of reinforcing efficacy based upon drug self-administration trial frequency with a series of prototypical stimulants **(Figure 1).**, and upon drug self-administration lever pressing response rate with a series of prototypical sedatives **(Figure 2)**, both as a function of dose.

Comparisons between self-administration drugs with respect to their reinforcing functions however, have continued to present methodological challenges to the extent that effects upon response topography (e.g., disruption of motor control) may compromise instrumental performance measures (e.g., lever

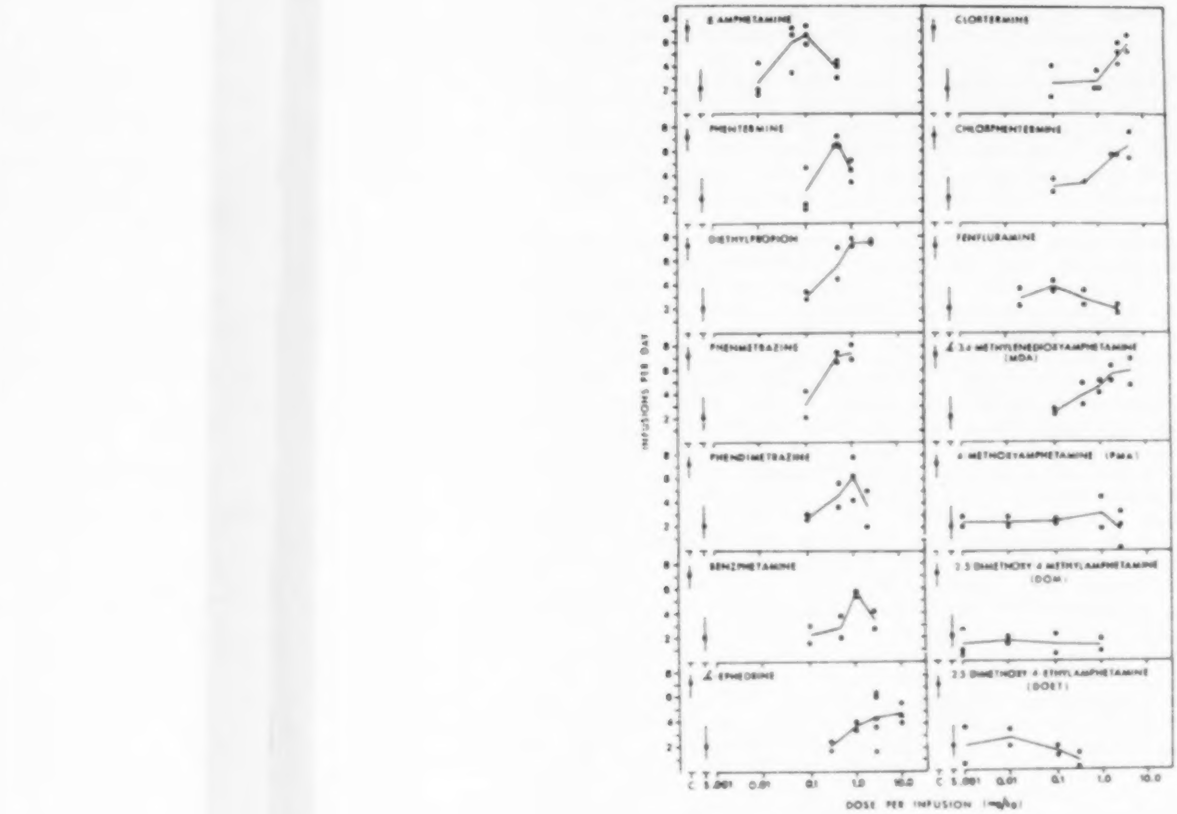

Figure 1. Average number of injections per day with 14 phenylethylamines. Intravenous injections were delivered upon completion of 160 lever presses: a 3 hour time-out followed each injection permitting a maximum of eight injections per day. C indicates mean of all the 3-day periods with cocaine which immediately preceded every substitution of a phenylethylamine or saline. S indicates mean of day 8-12 after substitution of saline (two saline substitutions in each of 15 animals). Brackets indicate ranges of individual animal's means. Drug data points indicate mean of days 8-12 after substitution of a drug for individual animals. Lines connect means at indicated doses of drug.

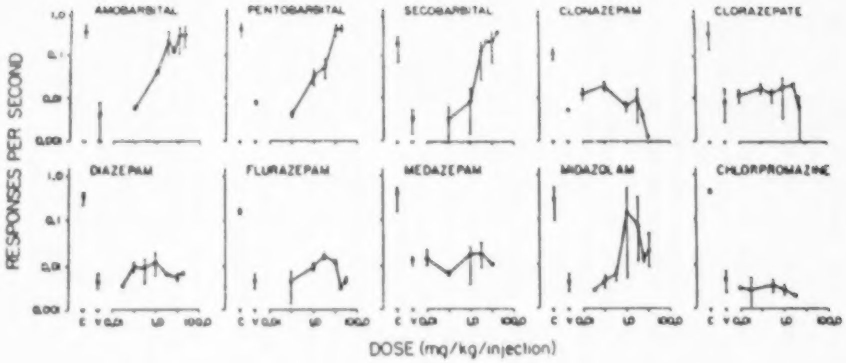

Figure 2. Mean response rates maintained by drug. Y-axis: responses per second, log scale. X-axis: dose (mg/kg/injection), log scale. For each animal exposed to a drug dose or vehicle (V), the overall response rate was calculated for the last 5 days of the condition by dividing the total responses (excluding those emitted during time-out) by the total time in seconds (excluding time-out periods). The mean and range of these overall response rates are presented. Control cocaine data (C) were calculated identically from the 3-day control periods which immediately preceded substitution of vehicle or the drug doses.

pressing), per se. A range of investigative approaches to th
solution of this problem has been reported including the use o
concurrent schedule procedures (Iglauer, *et. al.*, 1975), choic
precedures (Griffiths, *et. al.*, 1976; Johanson and Schuster
1977), response rate analysis (Balster and Schuster, 197
Griffiths, *et. al.*, 1981), and progressive ratio procedure
(Yanagita, 1973; Brady, *et. al.*, 1975; Brady and Griffiths
1976). The most extensively documented results have involved th
use of progressive ratio methods for measuring the relativ
reinforcing strength of a drug by determining the maximum amoun
of responding (i.e., work) that can be maintained by th
compound (Brady, *et. al.*, 1982). The response requirement i
systematically increased until the number of completed injectio
trials falls below a criterion level. Such "breaking point
determinations, for example, have been made over a range o
doses with fenfluramine, chlorphentermine, diethylpropion, an
cocaine (Griffiths, *et. al.*, 1978a). Within-animal comparison o
the maximum breaking points maintained by the different dru
are illustrated in *Figure 3* which shows that cocaine generall
maintained by the highest breaking points, followed in order b
diethylpropion, chlorphentermine, and fenfluramine.

A useful procedure for ranking the abuse liability of anorecti
drugs has also been described (Griffiths, *et. al.*, 1978b) base
upon an ordering of compounds in terms of a ratio between tw
doses: a dose that produced a specified anorectic effect and
dose that produced a specified reinforcing effect. Clearly, th
most desirable anorectic drug would have potent anorectic pro
perties but minimal reinforcing functions. An undesirabl
anorectic would be a weak appetite suppressant but a poten
reinforcer. A number of substituted phenylethylamines an
cocaine have been evaluated using this anorectic/reinforcemen
ratio (akin to the "therapeutic ratio", for example) and *Figur*
4 illustrates the results of such comparisons based upon bot
animal and human anorectic dose determinations.

Significantly, the generality of these results reported wit
animal self-injection models of abuse liability has been wel
documented in controlled laboratory drug self-administratio
studies with human volunteers. A broad range of pharmacologi
agents including heroin (Mello, *et. al.*, 1981), methadon
(McLeod, *et. al.*, 1982; Stitzer, *et*. *al.*, 1983), ethanol
(Griffiths, *et*. *al*., 1974; Mello, *et*. *al*., 1971). pentobarbita
(Griffiths, *et*. *al*., 1979), diazepam (Griffiths, *et*. *al*., 1983)
cocaine (Fischman and Schuster, 1982), amphetamine (Johanson an
Uhlenhuth, 1980 a,b), nicotine (Griffiths, *et*. *al*., 1982), an
marijuana Mendelson, *et*. *al*., 1976), among others (Chait, *et*.
al., 1984), has been shown to maintain drug self-administratio
by humans independently of whether the participant volunteer
were occasional or casual drug users (Johanson and Uhlenhuth
1980 a), had substantial and varied drug use histories (Fischm
an, 1976), or were physically dependent on drugs (Mello, *et*.
al., 1981; Stitzer, *et. al.*, 1983).

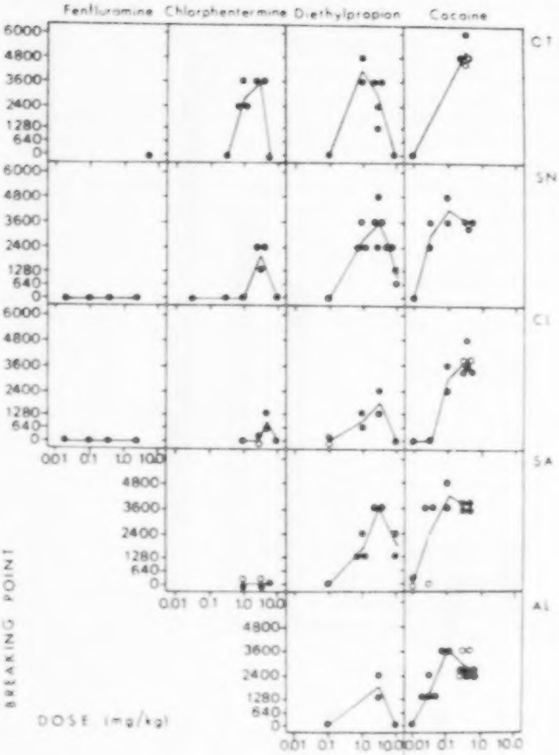

Figure 3. Breaking point values for doses of fenfluramine, chlorphentermine, diethylpropion, and cocaine in five baboons. Ordinates: Breaking points; Abscissae: Dose (mg/kg/inj.) Each point represents a single breaking point observation. Lines connect the means of the breaking point observations at different doses of drug. Filled circles indicate data obtained during the first exposure to a drug dose. Unfilled circles indicate data obtained during a second exposure to a drug dose.

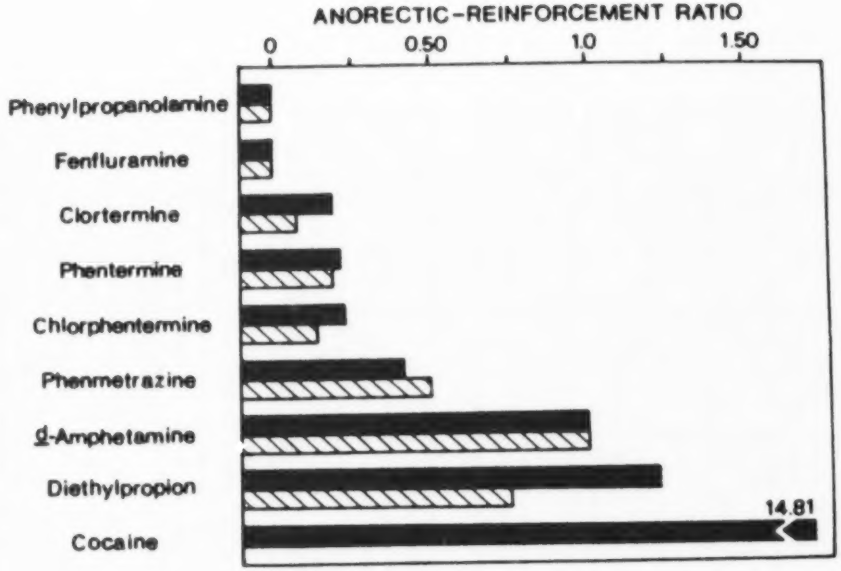

FIGURE 4. Anorectic-Reinforcement Ratios for cocaine and eight anorectic drugs. Filled bars show data derived entirely from baboon experiments. Striped bars show data derived from both human clinical information and baboon experiments. Compounds with high ratio values are more potent reinforcers (relative to their anorectic potency) than compounds with lower ratio values.

In other respects as well, the human data from drug self-administration studies in experimental settings tend to confirm the functional relations described in laboratory animal investigations (Bigelow, et. al., 1976). When "response cost" was manipulated, for example, with human inpatient volunteers self-administering their maintenance dose of methadone contingent upon an operant performance, introduction of a progressive ratio requirement for methadone decreased response rates and increased the likelihood that the subjects would detoxify (McLeod, et. al., 1983). Similarly, response cost studies with humans have shown that alcohol consumption levels varied inversely with the amount of work required to maintain drug access (Mello, et. al., 1968). And the reported data from experiments using choice procedures with human volunteers to assess the relative reinforcing strength of drugs has been generally consistent with the animal laboratory findings: pentobarbital is preferred to diazepam (Griffiths, et. al., 1980); high doses of cocaine are chosen over lower doses (Fischman and Schuster, 1979); and cocaine is preferred to procaine (Fischman, 1982).

While reinforcing functions as reflected by self-administration have provided a dominant focus for procedural approaches to the assessment of abuse liability over most of the last two decades, recent refinements in the analysis of discriminative stimulus functions have provided a more comprehensive basis for characterizing a drug's spectrum of action and evaluating its abuse liability (e.g., Schuster and Balster, 1977 a,b: Brady and Fischman, 1985). In addition, assessment procedures have been further enhanced by analysis of the behavioral effects, both acute and chronic, that follow as a direct consequence of drug administration. These eliciting stimulus functions have long provided a major focus for evaluating the biochemical and physiological dependence producing properties (i.e., tolerance and withdrawal effects) of drugs (Deneau, 1956; Jasinski, 1977; Seevers, 1936; Wikler, 1976). But the measurement of such direct behavioral effects has added an important dimension to the assessment of abuse liability as well. Substances with only minimal if any, disruptive behavioral affects are generally not regarded as drugs of abuse (e.g., caffeine in tea, coffee, or coca cola). In contrast, compounds self-administered even sparingly that are associated with behavioral decrements are considered to be highly abusable (e.g., lysergic acid diethylamide, LSD). Drugs may fall anywhere on the continua defined by these interacting parameters and a comprehensive evaluation of such behavioral toxicity can contribute importantly to the assessment of abuse liability. Procedures are now available (Brady, et. al., 1979; Heinz and Brady, 1980; Hienz, et. al., 1981), for example, to operationalize and quantify these relationships and provide a comparative analysis of the interactions between the disruptive eliciting effects of abused drugs upon sensory/motor functions, on the one hand, and their reinforcing functions in maintaining self-administration, on the other. This reinforcement/toxicity ratio (Brady, et. al., 1983; Brady, et. al., 1984) compares the relative potency of a drug as a reinforcer with its relative potency in eliciting sensory/-motor decrements and provides a quantitative comparative assessment of the relationship between drug self-administration and behavioral disruption. Figure 5 illustrates the results of such a relational analysis with a range of sedatives, stimulants, and dissociative anesthetics.

All of which is by was of emphasizing that there are now several converging lines of evidence--convergent operations, if you will--that testify to the reliability and broad generality of observations confirming the reinforcing functions of drugs. Morever, the orderliness of the data relating these reinforcing functions to independently derived measures of pharmacological activity (e.g., the anorectic/reinforcement ratio, the reinforcement/toxicity ratio, etc.) speaks directly to the issue of construct validity in evaluating the contribution of drug self-administration methods for the assessment of abuse liability. The issue of predictive validity on the other hand, continues to plague our most conscientious efforts largely because it remains unclear as to just how the purported phenomena to be predicted-drug abuse - is appropriately identified, defined, and measured in the natural ecology. Despite somewhat differing opinions in these regards, however, self-administration performances are clearly recognized to be prominently common feature of both alcohol and drug abuse.

The validity of laboratory self-administration assessment procedures however, is not to be judged on the basis of predicting "drug dependence" (i.e., tolerance and abstinence) nor even "drug abuse" (i.e., drug seeking and drug taking plus behavioral toxicity). Rather, their predictive validity resides in the evaluation of "abuse liability" (i.e., the likeihood that a given physiologically or behaviorally toxic substance will maintain drug seeking and drug taking under at least some conditions). An extensive array of chemical substances have abuse liability under some conditions within the framework of this definition, and an evaluation of the reinforcing functions of drugs provides information about at least this one prominent aspect of abuse liability assessment. To this extent, laboratory drug self-administration procedures can be seen to have "face validity", at the very least. They not only tell you that there are at least some conditions under which a drug can be self-administered (i.e., evidence of "inherent" reinforcing functions, if you will), but they can as well define the range of those conditions to a significant extent (i.e., different species of organism, dose dependence, etc.) and the influence of circumstances (i.e., environmental contingencies, deprivation, etc.) in potentiating or attenuating such reinforcing functions.

Finally, to provide a fitting "Come-to-Jesus" conclusion to this all-too-familiar "three-little-bears" story, let me add my equally tiresome "broken-record" commentary on the deplorable state of the nomenclature in this muddled arena of drug and alcohol "addiction", "dependence", "abuse" or whatever! The

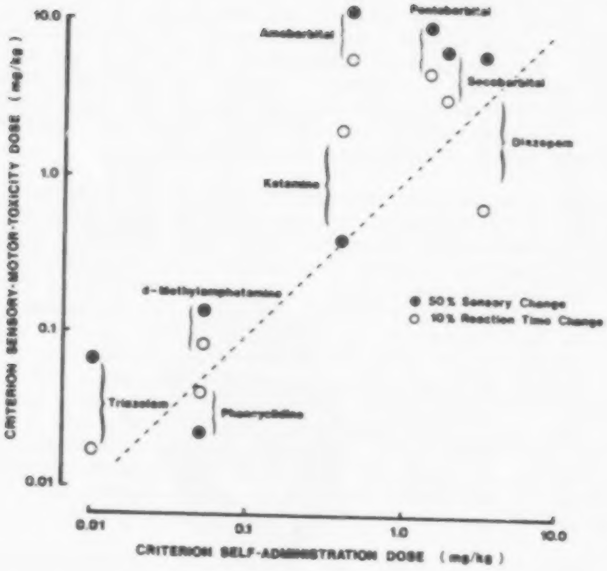

Figure 5. Relationship between criterion sensory and motor toxicity doses and criterion self-administration doses for three barbiturates, two benzodiazepines, two dissociative anesthetics, and a stimulant. The broken diagonal line represents equality between reinforcing and toxic doses.

interchangeable, quasi-technical use of these terms as referents for a bewildering range of phenomena and experimental pseudophenomena continues to produce a degree of semantic and taxonomic confusion that is perpetuated in even the most current and authoriative treatments of the subject by friends and relatives alike (Johanson, et. al., 1987). The terms themselves, persistently reified as substantive noun "things" that enter into subject-predicate relationships with other "things" (and affect, as well as are in turn affected by these other "things"), are seldom accorded appropriate conceptual status as constructs emerging from observed relationships between specifiable antecedents (biological and social) and definable consequences (biochemical, physiological, and behavioral). Within this relational context, the analysis of interacting biological and behavioral events would seem to provide a basis for defining these constructs more operationally and specifying the conditions under which a unifying conceptual framework can be developed for this prominent aspect of substance use and misuse.

At least some definitional clarity can be attained by dividing the vast array of events that characterize this domain into two reasonably exclusive categories based upon explicity operational criteria. Such a division is possible, for example, by distinguishing between events that occur before and events that occur after actual substance intake. As a first approximation, the defining operations of the "before" class, on the one hand, would include (but are not necessarily limited to) proactive drug-seeking and drug-discrimination behaviors. The defining operations of the "after" class, on the other hand, would focus upon the reactive biochemical, physiological, and behavioral changes associated with tolerance and an abstinence syndrome when drug is withdrawn. Figure 6 illustrates schematically (courtesy of my fellow iconoclast, Scott Lukas) this characterization of the drug dependence and abuse universe.

The temporal ordering of biochemical, physiological, and behavioral changes in relationship to the drug intake event can thus provide an operational basis for characterizing the range of a pharmacologic agents functional properties and for identifying distinguishable features of its spectrum of action. The relevance and importance of this distinction between proactive "abuse liability" and reactive "physiological dependence potential" resides in the fact that, from the perspective of drug assessment - the topic to which this essay is addressed- their defining properties are not coextensive, they do not invariably occur together, and the methods by which they are evaluated differ. Proactive drug-seeking and drug self-administration performances as cardinal signs in the assessment of abuse liability for example, can be maintained in strength by use patterns and doses of compounds (e.g., cocaine) that produce no significant tolerance or withdrawal - the reactive changes traditionally associated with physiological dependence. On the other hand, there are tolerance and abstinence-producing compounds (e.g., propranolol) that neither generate not maintain

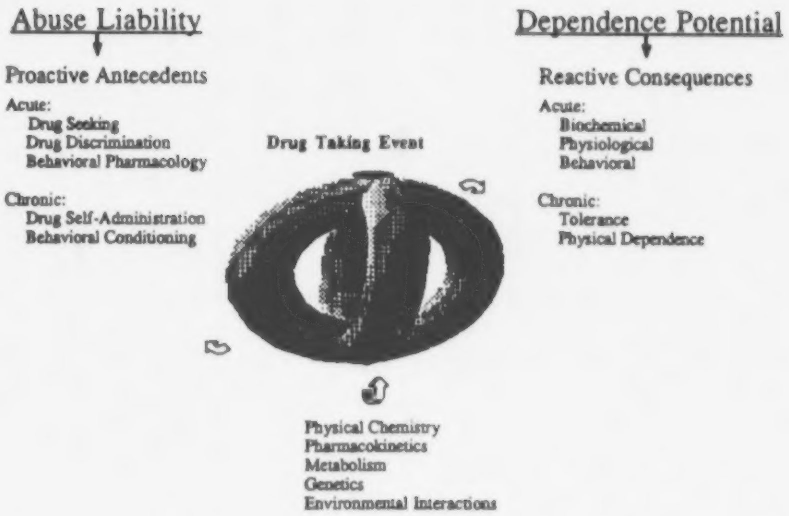

Figure 6. Schematic Characterization of Abuse and Dependence Processes.

drug self-administration.

Interactions between these proactive and reactive features of the drug and alcohol scene are of course, commonplace. Changes in drug-seeking and drug-taking often occur as sequelae to both the acute effects of a pharmacologic agent and to the tolerance and withdrawal effects that follow more chronic drug exposure. Conversely, the chemical and biological changes that define physiological dependence can as well be sequelae to the repeated self-administration of abused drugs. But the relative contributions of these distinguishable processes to drug and alcohol-related problems can vary with different pharmacological agents as a function of dose, environmental circumstances, and previous drug history (Mendelson and Mello, 1982).

Despite these interactive features however, it is important in confronting the validity questions at issue in this discussion to recognize that the methods used in both laboratory animals and humans to assess these distinguishable pharmacological properties are quite different. Not surprisingly, the predictive validity of self-administration as a drug evaluation procedure may be quite poor if the objective is to assess a compound's physiological dependence potential. But if the abuse liability of a substance as defined by the likelihood of it supporting drug-seeking and drug-taking, is to be evaluated, an assessment of its reinforcing functions by self-administration is clearly the method of choice.

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A Critique of Drug Self-Administration as a Method for Predicting Abuse Potential of Drugs

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The Committee on Problems of Drug Dependence has participated in an effort to assist federal regulatory and enforcement agencies in their effort to minimize problems of drug abuse by precluding the introduction of drugs with a high potential for abuse into extensive medical usage and illicit drug traffic. Drugs of high abuse potentiality are identified and their availability is delimitated. This endeavor has required the close cooperation between the scientific community and the regulatory agencies. Some of the major factors in these interactions has been the rapid development of the pharmaceutical industry, the rapid evolution of new drugs which act on the CNS, and the growing understanding of brain function. Despite the rapid development of the neurosciences and the understanding of psychopathology, the views of drug abuse have remained much the same over the last five or six decades. These views consist of the following propositions:

(1) A drug which shares a pharmacology with an abused drug has a similar potential for abuse. This proposition has a great deal of face validity even though it is not readily reconciled with a large body of epidemiologic data concerning the relative abuse of a number of narcotic analgesics, sedative-hypnotics, antianxiety drugs, and stimulants. There has been the view that these discrepancies can be explained by nonpharmacologic factors such as prevailing attitudes, the disposition of the drug culture, and drug availability.

(2) In 1931, Tatum and Seevers introduced the concept of drug-induced dependency as an important dimension of drug addiction based on the demonstration of physical dependence on narcotic analgesics in animals. The concept of physical dependence obtained vogue as an explanation for continued and compulsive use of drugs because the discomforting signs, symptoms, and feeling states of both acute and protracted abstinence could be relieved by drugs of abuse. However, we were soon reminded that the production of physical dependence was not a necessary condition for the abuse of many drugs. Thus, LSD-like drugs, and marijuana, when used chronically in moderate

but abused doses, do not produce a readily measurable abstinence syndrome. Furthermore, the chronic administration of certain opioid antagonists, notably nalorphine and cyclazocine, induce physical dependence; however, their abstinence syndromes are not associated with drug-seeking behavior.

(3) A third pharmacologic framework from which drug abuse has been viewed is that drugs of abuse produce subjective effects which are pleasant and reinforcing. The remainder of this chapter will be concerned with the validity of this proposition and with methods used to measure these subjective states.

The abuse potential of a drug has been assessed by showing it to be pharmacologically equivalent to a standard drug that is known to be abused. Techniques for establishing pharmacologic equivalence included studies, in both animals and man, which utilized tolerance, dependence, cross-tolerance, suppression, and direct addiction methodologies. Other estimates of abuse liability of drugs have been made using drug self-administration substitution procedures in animals. Relative reinforcing efficacy can also be determined by comparison with standard drugs of abuse using progressive ratio and choice procedures. Although drug discrimination paradigms provide valuable data they will not be discussed in this chapter.

The mechanisms underlying self-administration behavior in animals are not well-understood. It is believed that self-administration behavior is a particular case of operant behavior, that is, behavior controlled by its consequences, and that certain drugs are positive reinforcers. It is believed that drugs of abuse are self-administered because they produce pleasant subjective effects which increases the future occurrence of their self-administration. It is usually assumed that humans self-administer drugs because they "like" the effects of the drugs. Fraser et al., (1961), Martin et al., (1971), and McClane and Martin (1976), have reported that addicts show a dose-related increase in the degree of liking of opiates, amphetamines, and barbiturates. With respect to subject's liking in a study of five amphetamines, the order of potency (highest to lowest) was methamphetamine, amphetamine, methylphenidate, phenmetrazine, and ephedrine (Martin et al., 1971). This order of potency corresponds well with the animal literature; however, it should be noted that while ephedrine is capable of producing positive subjective effects in humans of the same magnitude as amphetamine, the incidence of ephedrine abuse currently is probably very small.

The agreement between self-administration data in several species and liking-data obtained in several patient populations is excellent for some classes of drugs but is poor for other classes of drugs. Probably very nearly 100 percent of animals tested with cocaine will intravenously self-administer the drug. Rats, cats, dogs, squirrel monkeys, rhesus monkeys, and baboons all intravenously self-administer d-amphetamine as summarized by Griffiths et al., (1980). In addition, the results with amphetamine correspond well with the effects of amphetamine in humans with no prior chronic drug experience. In Lasagna's 1955 study, "amphetamine was outstanding in its ability to create a pleasant state" in normal individuals not in pain.

There is also a human/animal concordance with respect to the relative potencies of *d*- and *l*-amphetamine. In a comparative study in humans on maximum mood responses, the potency of d-amphetamine versus l-amphetamine was on the order of 1.5 to 1 and 3 to 1 (Smith and Davis 1977). These data correspond to amphetamine self-administration data in rats and in the rhesus monkey (Yokel and Pickens 1973; Balster and Schuster 1973).

There are many examples in the self-administration literature which support the contention that many drugs which are abused by humans are self-administered by animals: alcohol, nicotine, opiates, benzodiazepines, cocaine, sedative-hypnotics, amphetamines, and phencyclidine (Balster and Lukas 1985; Griffiths *et al*., 1985; Griffiths and Balster 1979). There is no doubt that self- administration techniques are powerful methods for measuring the central CNS effects of certain types of drugs. There are drugs, however, which are abused by humans that are not self-administered by animals and include LSD, mescaline, DOM and THC.

In one study using rhesus monkeys, Hoffmeister (1975) gave evidence that LSD, had negative reinforcing properties in drug-naive animals. Deneau *et al*., (1969) found that none of four monkeys initiated self-administration of mescaline either spontaneously or after programmed administrations. In contrast to the phenylalkylamine MDMA which is in fact self-administered (Lamb and Griffiths, 1987), the phenylpropylamine DOM (Griffiths *et al*., 1979), is not (Griffiths *et al*., 1979). In addition to DOM, various indolealkylamines such as psilocybin, psilocin and N,N-DMT appear to involve serotonin type 2 receptors or possibly tryptamine receptors; this difference from MDMA may be a basis why these latter compounds are not self-administered by animals. Finally, in contrast to reports that humans will work for marijuana and THC doses, rats and rhesus monkeys will only self-administer THC at very low levels. van Ree *et al*., (1978) observed self- administration of THC, 300 μg/kg/injection, in only 2 of 5 rats. Kaymakcalan (1973) found that programmed THC injections promoted THC self-administration in only 2 of 6 monkeys; however, Harris *et al*., (1974) did not find this maneuver to be successful in a total of 10 monkeys.

In another category, there are drugs which are not abused by humans that are self-administered by animals and include the following agents: ketocyclazocine, ethylketocyclazocine, apomorphine, piribedil, clonidine, procaine, N-allylnormetazocine, metazocine and phenylethanolamine. Ketocyclazocine or ethylketocyclazocine were self-administered by both morphine-dependent or drug-naive rats, but not by rhesus monkeys (Young and Khazan 1983; Collins *et al*., 1984; Tang and Collins 1985; Woods *et al*., 1979). This species difference raises important issues concerning the selectivity and specificity of the self-administration method, an issue which will be discussed subsequently. The DA 1 and DA 2 agonist apomorphine was self-administered by rats; the DA 2 agonist piribedil was self-administered by both rats and the rhesus monkey (Yokel and Wise 1978; Woolverton 1986). The alpha 2 adrenergic agonist clonidine is self-administered (Woolverton et al., 1982) but the alpha 1 agonist methoxamine is not in dogs (Risner 1975). The esteratic local anesthetic procaine is self-administered by rats and monkeys in the same pattern as cocaine self-administration (Collins et al., 1984; Ford and Balster 1977; Hammerbeck and Mitchell 1978). Three other short-acting esteratic local anesthetics, chloroprocaine, dimethylprocaine, and dimethocaine, also supported self-administration behavior in the rhesus monkey (de la Garza and Johanson 1982; Woolverton and Balster 1982). It is interesting to note that three human subjects identified large, (48 and 96 mg) doses of i.v. procaine as cocaine (Fischman et al., 1983). However, lower doses of procaine were like placebo on the ARCI and POMS scales. (+)-NANM and both (+) and (-)-metazocine supported self-administration behavior in rhesus monkeys, and these data bear on PCP self-administration (Slifer and Balster 1983; Slifer et al., 1986). In the dog, the endogenous compound beta-phenylethanolamine was self-administered (Shannon and Thompson 1984). Thus, self-administration studies in animals have shown a good ability to predict abuse potential for some drug classes but not for others. A number of factors can be identified which may contribute to the diversity of these results.

One complicating variable that relates to selectivity is species differences which has been alluded to and exemplified. Other examples are the differences between man, monkeys, dogs, rats, and mice in their responses to normorphine, benzomorphans and meperidine. In studies of maximally abstinent morphine-dependent dogs Martin et al., (1978) found that meperidine and normorphine did not suppress morphine withdrawal. It is also well known that there are marked differences in drug metabolism between species and that there may be active metabolites which may enhance or antagonize the reinforcing properties of the parent drug (e.g. ethanol and acetaldehyde; diazepam and nordiazepam). New knowledge about the nicotinic cholinergic receptor reveals species differences in the amino acid sequences of the receptor protein. How do these differences impinge upon the self-administration of nicotine across species and on the specificity of the receptor? The same questions may be asked of other drug receptors. And finally, the "wiring" of brain reinforcing systems and their neurotransmitter constituency may differ from species to species.

Between differences in individual and species pharmacologic and physiologic responses to drugs is paralleled by differences in behavioral measures. Although the monkey and the rat can be trained to self-administer opiates, it is much more difficult to do so in the dog. Jones and Prada (1973) found that 18 of 22 dogs decreased their rates of responding for morphine in doses ranging between 0.01 and 0.25 mg/kg/injection. Subsequently, only 1 dog in 10 was able to be trained to self-administer morphine. These data compare with the 1955 human study by Lasagna et al., showing the low incidence of "pleasant" reactions to the administration of morphine or heroin in a normal population. Although 7 of 14 addicts reported that 4 mg of heroin was "pleasant", 7 of 11 normal subjects reported that this dose of heroin was unpleasant. Likewise, 12 of 14 post-addicts reported that 15 mg of morphine was pleasant, while 8 of 11 normal subjects reported that this dose of morphine was "the most unpleasant medication". Thus, although morphine-like drugs appear to be reinforcing in opiate addicts, many normal subjects not in pain do not like the effects of opiates. These data raise an important issue: What is the relevant population for human studies? Drug-experienced? or drug-naive?

It is important in attempting to formulate general theories of drug abuse to relate animal and human behavior. In the United States estimates would suggest that no more than 15% of the population uses alcohol compulsively. A somewhat greater percentage, 30%, but nevertheless, a relatively small percent, smoke cigarettes. A smaller percent use cocaine, amphetamines and marijuana regularly, and the number of people in the U.S. that abuse opiates probably does not exceed 750,000, or about 0.3% of the population. These data compare with 30-day prevalence data from high school seniors of the class of 1985 (Johnston et al., 1986). Hence, the overall conclusion that one must draw is that in humans only a relatively small portion will use socially approved or socially disapproved euphoriants on a regular basis. A prudent conclusion from these sorts of considerations would be that the largest portion of human beings use euphoriants only occasionally, or not at all.

Because of the variability of subjective states produced by drugs in humans, the problem of validating methods for assessing the abuse potentiality of drugs in animals is a difficult one. How can the self-administration model be validated? First, as outlined by Martin (1977), there must be concordance between animal and clinical results. In order to define the limitations of methodologies, more effort should be placed on determining the reasons for discordant results. Secondly, there also must be some sort of concordance between both the incidence and perniciousness of street abuse on the one hand, and the results of both animal and clinical studies on the other. Because fads and trends play a major role in drug abuse patterns, a good concordance can be expected only for those drugs whose street abuse is well established. The following is an attempt at finding such concordances.

One quantitative estimate of the relative reinforcing strengths of drugs has been made by determining the highest number of responses that can be performed by the animal before self-administration performance diminishes to baseline control levels. This "progressive ratio" test entails the systematic increase of the response requirement until the subject fails to

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Table 1. Breakpoints of Drug Self-Administration Derived from Progressive Ratio Tests in Various Species

Author	Species	Drug	Mean Breakpoint
Yanagita 1976	rhesus	Cocaine	2850 (6400)
		Morphine	1608 (4800
		Amphetamine	1383 (3200)
		Ethanol	1050 (2400)
		Pentobarbital	967 (1600)
		Caffeine	458 (1600)
Griffiths *et al.*, 1978	baboon	Cocaine	3840 (4800)
		Diethylpropion	3120 (3600)
		Chlorphentermine	1820 (3600)
		Fenfluramine	0
Hoffmeister 1979	rhesus	Heroin	10667 (12800)
		Codeine	6400 (6400)
		Dextropropoxyphene	3733 (6400)
		Pentazocine	2267 (3200)
Griffiths *et al.*, 1975	baboon	Secobarbital	4800 (4800)
		Cocaine	4320 (4800)
		Pentazocine	1653 (2400)
Moreton *et al.*, 1977	rhesus	Ketamine	192 (256)
Risner and Silcox 1977	dog	Cocaine	1435 (3750)
		Amphetamine	652 (1350)
		Mazindol	650 (1350)
		Fenfluramine	15 (30)
Risner and Goldberg 1983	dog	Cocaine	2430 (3750)
		Nicotine	390 (510)

The data depicted are the mean break points for each drug used in each study. The numbers in parentheses are the highest ratios attained for that drug in the study.

Table 2. Correlations of Breakpoint Data from Drug Self-Administration Studies with DAWN Mentions from 1985.

Drug	Normalized Breakpoint	Mentions 1985		
		ER	ME	Deaths
Secobarbital	111	508	107	21
Cocaine	100	13501	643	152
Diethylpropion	81	-	-	0
Heroin/Morphine	56	16696	1315	353
Amphetamine	49	1118	77	8
Chlorphentermine	47	-	-	-
Mazindol	45	-	-	-
Methylphenidate	38	377	3	0
Ethanol	37	21090	1288	161
Pentobarbital	34	178	44	10
Codeine	34	1014	351	33
Dextropropoxyphene	20	1876	234	45
Caffeine	16	-	-	-
Nicotine	16	-	-	-
Pentazocine	12	617	10	0
Fenfluramine	0.5	-	-	-
Spearman Rank Correlation Coefficient		0.28	0.35	0.41

Breakpoint data for the drugs have been normalized, cocaine = 100. Emergency room (ER) and medical examiner (ME) mentions and deaths are from the 1985 DAWN data system. None of the correlations are significant; critical $(r_s)_{0.05\ (2),\ 14} = 0.54$

complete a ratio, i.e. until the "break-point" of the performance is reached. Breakpoints have been used to compare the relative reinforcement efficacies of drugs. Table 1 summarizes the available break-point data in animals for certain abused drugs. As can be seen in Table 2, there is a poor correlation between the breakpoint data from drug self-administration studies in animals, and the data from the DAWN system. The numbers of emergency room and medical examiner mentions as well as the number of deaths reported for the drugs listed were not significantly correlated with the order of drugs determined from progressive ratio tests. Thus, it would appear that the relative reinforcing efficacy of drugs as determined in animals is a poor predictor of drug use in humans. Although there are many caveats in this interpretation, the basic tenet of this conclusion is probably true.

We will turn now to the issue of whether drugs are especially reinforcing to certain populations. During the development of the ARCI and the validation of its subscales, it was observed that a certain constellation of items were associated in a dose-related manner with certain abused drugs. These items were designated as the Morphine-Benzedrine Group scale which measures feelings of popularity, efficiency, pleasantness, satisfaction, and ease in talking. Morphine-like drugs (Fraser _et al_., 1961), amphetamines (Martin _et al_., 1971), and barbiturates (Fraser _et al_., 1961) produced dose-related increases of MBG scores. These subjective effects of morphine which were reported by abstinent heroin addicts contrasted with those reported by Smith and Beecher (1962) in non-addict subjects. A list of somatic and physical activities characterizing morphine and heroin includes: itchy, nauseated, dizzy, blurry-eyed, drowsy, tired, sleepy, sluggish, weak, heavy-lidded, fuzzy-headed, mentally cloudy, mentally slow, groggy, dreamy and irritable. Furthermore, Hill _et al_.,(1962) as well as other investigators had observed that abstinent heroin addicts had elevations on the depression (D) scale of the MMPI. Although the prisoner addicts, who were experimental subjects on the wards of the ARC, reported a number of depressive symptoms, none had a depressive psychosis. That is, they did not feel worthless, they had a good sense of humor, were not impotent, did not have diminished libidos, had good appetites, and had few sleep problems. The negative feeling states that they reported seemed to be polarly opposite to many of the euphoric symptoms reported after the administration of morphine, amphetamine, and barbiturates. These negative feeling states were designated by Martin _et al_., (1977) as hypophoria and include feelings of unpopularity, ineptness and inefficiency, a negative perception of life, and of being unappreciated. These negative feelings are decreased by single doses of opiates, amphetamines and barbiturates (Hill _et al_., 1963; Haertzen 1966; Martin _et al_., 1971; Martin _et al_., 1974). A questionnaire was devised which not only measured feelings of hypophoria but also feelings related to egocentricity, impulsivity, need, and sociopathy. The combined score of these subscales was called the ARC Maturation Scale and measures immaturity. The first study employing this scale examined groups of prisoner addicts, alcoholics who had sought treatment, and a group of students and faculty of a theologic seminary (Martin et al., 1977; Hewitt and Martin 1979; Martin et al., 1978). These data show that there is a highly positive correlation between alcoholism, narcotic abuse, and other antisocial behavior, and scores on the ARC Maturation Scale and its subscales.

Thus, the reinforcing properties of drugs of abuse may be especially strong in subjects who have a high degree of hypophoric feelings. Whether there are animal models of hypophoria, or whether animal models can be developed, is not known at the present time. It may be that affective state (e.g. hypophoria and depression) is a critical dimension which predisposes individuals to drug abuse. It is important to realize that the ability of a drug to transform feelings of hypophoria into feelings of well being is not necessarily a pernicious property; rather, it may have practical therapeutic value. Early studies by Martin suggested that hypophoria was negatively related to age. More recent studies support these findings. The high vulnerability of adolescents to the use and abuse of drugs probably subsides with age in most people. Although identifying the positive reinforcing properties of drugs may serve a useful exercise in preventive medicine, it has not provided an adequate solution to the pernicious and toxic properties of drugs of abuse. It is our opinion that the results of screening methods for identifying reinforcing properties of drugs should not be overinterpreted, and that these properties may provide valuable clues for identifying drugs of therapeutic value.

A complete reference list is available from the authors.

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Biological Evaluation of Compounds for Their Physical Dependence Potential and Abuse Liability. XI. Drug Testing Program of the Committee on Problems of Drug Dependence, Inc. (1987)

A. Jacobson

The analgesic testing program of the Committee on Problems of Drug Dependence (CPDD) has been concerned with the biological evaluation of compounds for their physical dependence potential and abuse liability for more than 37 years. Although the name of the Committee was modified from the "Committee on Drug Addiction and Narcotics" (of the National Research Council, in the National Academy of Sciences), at least one of the Committee's main purposes has remained constant over almost four decades - to provide scientific information about a drug's potential for abuse, as a public service. For the past several years the CPDD has attempted to find procedures and laboratories which would be adequate to provide information about two other drug classes, the stimulants and depressants. This year, the groups involved with the stimulant/depressant program examined a number of compounds at the request of the World Health Organization, and provided the obtained information to that organization under the auspices of the CPDD. Unlike the analgesic testing program, however, the stimulant/depressant program has not, as yet, been opened for examination of compounds submitted from the two other groups which have been noted to be part of our constituency (Jacobson, 1987), the universities and the pharmaceutical industry.

ANALGESIC AND STIMULANT/DEPRESSANT TESTING LABORATORIES

Three groups are concerned with the evaluation of compounds as analgesics: the Medical College of Virginia (MCV) (Drs. M. Aceto, L. Harris, E. May, and E. Bowman), the University of Michigan (UM) (Drs. J. Woods, F. Medzihradsky, C. Smith, G. Winger, and D. Gmerek), and NIDDK, NIH (Dr. A. Jacobson and M. Mattson). The complete work of the MCV and UM groups is published in this monograph (Aceto et al. 1988; Woods et al. 1988), and should be consulted for detailed information on the evaluated compounds.

Five groups have been involved with the evaluation of the stimulant and depressant classes of compounds: the University of Chicago (Drs. C. Johanson, S. Evans, and K. Grant), the Medical College of Virginia (Drs. G. Patrick, L. Powell, and L. Harris), the Johns Hopkins University (Drs. R. Griffiths, N. Ator, and J. Brady), NIDDK, NIH (Dr. A. Jacobson, M. Mattson), and NIDA (Dr. E. Cone). The report from the stimulant/depressant laboratories has been submitted to the CPDD and the World Health Organization. It was prepared by a subcommittee (Drs. C. Johanson, G. Patrick, L. Harris and A. Jacobson), and it is included in this paper.

DRUG TESTING PROGRAM STATISTICS

The number of compounds sent to MCV and UM has varied considerably over the past nine years. This year (5/1/86 to 4/30/87) the number of compounds which were sent to MCV and UM was 49 and 40, respectively, within the standard deviation of the mean for the previous eight years. There has been a change in the procedures of the two groups. MCV has undertaken all of the single dose suppression and precipitated withdrawal studies in the rhesus monkey for new compounds, and has continued to examine compounds by rat infusion and in their antinociceptive assays (tail flick and PPQ), and the assay for narcotic antagonist activity (TFA) in mice. UM has continued their study of the effect of the new compounds in vitro (rat brain membrane binding studies, and in the electrically stimulated mouse vas deferens) and, when desired, in drug discrimination and self administration in the rhesus monkey. Both groups will continue to study compounds in primary physical dependence in the monkey. My group at NIH, NIDDK, studies the antinociceptive properties of the compounds in the hot plate assay in mice, as well as some in vitro assays when indicated. The number of reports which will be included in this year's Annual Report from MCV and UM are either slightly above the mean, or very near the mean, respectively, of the number included in their reports in the past 8 years.

We have seen a continued diminution of the number of compounds submitted from industrial sources in the US (3% of the total number of compounds received for evaluation from 5/1/86 to 4/30/87); however, submissions from foreign industry (12%) has remained at about the mean of the percentage determined for them over the previous 8 years (10%). We have also seen a slight decline in the number of compounds submitted by US universities (27% - the mean is 33% ± 9%), and a decided increase in the number of compounds submitted by foreign universities (32% - mean of 14% ± 11%). The NIDA/DEA series of fentanyl-like compounds which were examined this year was extensive (22% of the total number), well over the mean of the number usually received from that source (2%). The total number of compounds we have examined this year increased considerably (by ca. 26%) over the mean of the number submitted during the previous three years. Thus far, as one source declines, another increases. Overall, 59% of the examined compounds originated in university laboratories, 15% were from industrial sources, and 25% were from US governmental agencies. It should be mentioned that the standard deviations of the mean of all of these sources are very large; the percentage from any one source has undergone considerable fluctuation over the last several years. Nevertheless, it is obvious that there has been a statistically significant decline in US industry interest in narcotic analgesics over the past decade, but not of foreign industry interest.

CLASSES OF OPIOID-LIKE COMPOUNDS, AND SPECIFIC COMPOUNDS OF INTEREST AMONG THE EXAMINED OPIOIDS

The 72 compounds which have been evaluated as analgesics in the reports from MCV (Aceto et al., 1988) and UM (Woods et al., 1988) have been distributed, according to their structural similarity, among nine tables appended to this report. The compounds are conveniently classified as the 4,5-epoxymorphinans (tables 1 and 2), the morphinans and 6,7-benzomorphans (table 3), and methadone-like and pethidine-like compounds (table 4). The pethidine-like compounds are continued in table 5, with the considerable number of fentanyl-like compounds in tables 6 and 7. Compounds which are not as easily labelled by a conventional class are shown as the miscellaneous compounds in tables 8 and 9. Structure-activity relationships are, perhaps, more easily seen when compounds are grouped in this way, and the evolution of the structural types can be discerned by comparison with those shown in my previous reports (Jacobson, 1987).

The CPDD has been examining 4,5-epoxymorphinans for the past four decades. Heroin, morphine, codeine, nalorphine, naloxone and naltrexone are classified as 4,5-epoxymorphinans. This year we have a remarkable group of six new compounds in that class (NIH 10420, 10426, 10427, 10443 - 10445 in table 1), submitted by our pharmaceutical industry constituency. Some of these compounds have a very long duration of action as narcotic antagonists, others show potent agonist and antagonist activity. The activity of some or all of these compounds may be due to their interaction with various opioid receptors in an irreversible manner. That is, they may be acting as affinity ligands which, unlike most other affinity ligands, easily access the brain on parenteral introduction. For example, pretreatment of a non-dependent rhesus monkey with 0.35 mg/kg of NIH 10445 blocked the acute effects of morphine for two weeks (Aceto et al., 1988). NIH 10420 clearly substituted for morphine in the SDS assay and had morphine-like potency in the PPQ assay, it showed weak antagonist properties in precipitated withdrawal studies - two monkeys were in withdrawal for 3 days, even after morphine was given to them (Aceto et al., 1988). NIH 10426 was a mixed agonist-antagonist on the mouse vas deferens. It displayed fairly potent antagonist actions to U50,488, suggesting selectivity for kappa opioid receptors (Woods et al., 1988). Two of these compounds appeared to be fairly "pure" antagonists, NIH 10443 and 10444. All of the six compounds have a cyclopropylmethyl group on the nitrogen atom and a 6-keto moiety, as in naltrexone. Half of the six had a phenolic hydroxyl, the other half a methoxy group on the aromatic ring, as in codeine. The difference between these compounds and former 4,5-epoxymorphinans lies in the C-14 position. Naltrexone has a C-14 hydroxyl group, but these compounds have para-substituted cinnamoylamino moieties at C-14, the para substituent being either bromine, chlorine or a methyl group. An amide at the C-14 position is a little unusual. However, Dr. S. Archer's group (Archer et al., 1985) prepared chloroacryloyl amides (NH-CO-CH=CH-Cl) at the C-6 position in structurally similar compounds and found them to be good Michael acceptors (i.e., potential affinity ligands).

The industrial constituency has also provided us with a potent compound which was classified among the miscellaneous compounds (NIH 10412, table 8), although it somewhat resembles to 4,5-epoxymorphinans. It is a potent agonist-antagonist. It clearly indicates the evolution of that class of analgesic.

A structurally interesting group of compounds is being prepared by the second of our constituencies, the university group. NIH 10500, shown in table 3, is based on the morphinans. It is reminiscent of the Diels-Alder products which gave us etorphine and diprenorphine. However, the new ring formed beta, rather than alpha, between C-6 and C-14, providing a beta C-7 side-chain, rather than the usual alpha as in etorphine. These compounds should, in the future, provide us with information about the conformational requirements of the opioid receptors for those rings. At the moment, such compounds pose complex problems in nomenclature. We should have further biological information about that series next year. The university constituency has also given us compounds resembling PCP structurally (NIH 10531, table 5), but which seem to have pethidine-like, rather than PCP-like, activity (see, also, NIH 10012-10014 in table 4). These compounds do not bind appreciably to the PCP receptor. A re-examination of pethidine itself (NIH 10522 or 5221, table 4) exemplifies our ability to replicate experiments, and provides base-line information for comparison with the pethidine-like bicyclo compounds, NIH 10479, 10480, 10481, 10529 and 10530 (table 9), some of which had agonist activity comparable to, or greater than, pethidine in our mouse antinociceptive assays.

Two university compounds examined this year, NIH 10399 and 10400 (table 2), are quite similar to the aforementioned Archer et al. (1985) series of compounds. They are potent narcotic antagonists. The data provided to the university groups, most of which are supported by NIDA grants, will eventually be published by these groups with acknowledgement to either MCV or UM and the CPDD for the provision of data.

Lastly, a governmental unit submitted a considerable number of fentanyl-like compounds. Our data enabled them to proceed with the scheduling of the compounds. The structures of the compounds are shown in tables 6 and 7 (NIH 10468, 10482-10491, 10493, 10505-10506, and 10538). Some of the compounds are 50 to 100 times as potent as morphine in the SDS assay in monkeys, and in the mouse antinociceptive assays. It should be noted that the university constituency is also submitting fentanyl-like compounds (NIH 10476-10478, in table 6) which are fairly potent in the mouse antinociceptive assays.

STIMULANT/DEPRESSANT EVALUATION

At the request of the World Health Organization (WHO) the Committee on Problems of Drug Dependence (CPDD) agreed to undertake the evaluation of the dependence potential of six compounds proposed for review of possible control under the 1971 Convention on Psychotropic Drugs. The drugs were supplied to the CPDD by the International Federation of Pharmaceutical Manufacturers Associations (IFPMA). Of the six original drugs, one was unavailable and a second was provided both as a free alcohol and as a carbamate.

The compounds were assigned for evaluation to the CPDD Stimulant/Depressant Testing Group. I served as the coordinator at NIDDK, NIH, and the laboratories at the University of Chicago, Johns Hopkins University and Virginia Commonwealth University served as the biological testing sites. All compounds were tested by code number and the laboratories were blind as to the structures until the report was prepared. Due to pressures of time, all tests on the drugs were not completed at the time of the initial report. Subsequent data were provided for the report to the Expert Committee (not included herein). One drug proved to be so unstable in aqueous solution as to preclude many tests (stability studies were carried out in a NIDA laboratory).

A summary of the data obtained for the six compounds can be found in table 10 along with a number of control drugs. The test procedures were desigred to give some information concerning the pharmacological resemblance to known drugs, their physical dependence potential and the reinforcing effects of the compounds. The results will be presented in this order for all of the drugs tested.

CPDD 0011, 3-Methyl-1-pentyn-3-ol (Methylpentynol, meparfynol)
Methylpentynol, like the barbiturates and benzodiazepines, is active in the inverted screen tests and depresses spontaneous activity. In drug discrimination procedures, the drug was generally recognized as a barbiturate. In physical dependence studies, the drug partially substituted for pentobarbital in dependent rats and exhibited barbiturate-like abstinence signs on withdrawal. The fact that the drug may produce physical dependence was confirmed in one monkey in the self-administration study. Methylpentynol is readily self-administered indicating that it has positive reinforcing properties. In all of these tests, the drug has between 1/5 and 1/8 the potency of pentobarbital.

CPDD 0011A, 3-Methyl-1-pentyn-3-ol-carbamate (Methylpentynol carbamate, Meparfynol carbamate, etc.)
This carbamate analog of methylpentynol is active in the inverted screen test. Its effect on spontaneous activity is one of slight stimulation. This is often seen with low doses of barbiturates. Higher doses of this compound were precluded by toxicity. Drug discrimination studies are in progress but have been impeded by toxicity. Like the parent compound, the drug partially substituted for pentobarbital in dependent rats and exhibited barbiturate-like abstinence signs on withdrawal. The reinforcing properties of this drug have not been tested due to limited supply of the compound. In the tests completed, this drug is about twice as potent as the parent compound.

CPDD 0012, 2-Methyl-4-(2,2,2-trichloro-1-hydroxyethoxy)-2-pentanol (Chloralodol, chlorhexadol).

This prodrug for trichloroethanol is active in the inverted screen test and in depressing locomotor activity, when administered in sesame oil. In drug discrimination studies, it was not identified as diazepam-like by rats trained on diazepam, when given in aqueous solution immediately upon dissolution. Due to the chemical instability of this compound on standing in aqueous solution, it has not been tested in the other test procedures. The compound appears relatively impotent, approximately 1/15 the potency of pentobarbital.

CPDD 0013, N-(Aminocarbonyl)-2-bromo-2-ethylbutanamide (Carbromal)

This drug may be metabolized to a barbiturate-like compound or to a bromide carrier. It is active in the inverted screen test, and produced mild stimulant effects on spontaneous activity in the same dose range. In drug discrimination studies it was identified as pentobarbital-like by the pigeon, and there was partial generalization in the pentobarbital-trained rhesus monkey. It has not been tested in diazepam-trained rats or baboons. Studies of physical dependence liability in the rat are in progress; the compound has not been tested in drug self-administration. The potency of compound CPDD 0013 appears to be in the range of 1/3 to 1/5 that of pentobarbital.

CPDD 0014, 5-(2-Chloroethyl)-4-methylthiazole ethanedisulfonate (Clomethiazole edisilate, heminevrin)

This compound exhibited activity in both the inverted screen test and depression of spontaneous locomotor activity. In drug discrimination studies, it was identified as pentobarbital-like by the pigeon, but it did not generalize in pentobarbital-trained rhesus monkeys, diazepam-trained rats or lorazepam-trained baboons. In physical dependence studies in the rat, the compound exhibited only slight substitution in pentobarbital-dependent animals. Signs of abstinence were not seen following discontinuation of the drug. The drug appears to be reinforcing in that it was self-administered by rhesus monkeys. Compound CPDD 0014 is approximately 1/3 to 1/5 as potent as pentobarbital.

CPDD 0016, 2,2,2-Trichloroethanol dihydrogen phosphate (Triclofos)

This trichloroethanol prodrug was active in both the inverted screen test and in depression of spontaneous activity. It was identified as diazepam-like by the rat in discrimination studies, but did not generalize in lorazepam- or pentobarbital-trained baboons. Drug discrimination studies in pentobarbital-trained pigeons and rhesus monkeys are in progress. In physical dependence studies in the rat, the high dose substituted for pentobarbital, and a mild abstinence syndrome was observed following withdrawal. The compound has not been tested in self-administration procedures. CPDD 0016 is rather weak, with potency in the range of 1/15 to 1/20 that of pentobarbital.

Standard Compounds

The laboratories also have evaluated additional compounds generally under blind conditions that could be considered standards for the test compounds. These data are included in this report for purposes of comparison.

Meprobamate (CPDD 0018)

Meprobamate is active in the inverted screen test and in depressing locomotor activity. In drug discrimination studies, it substitutes for diazepam in the rat and for pentobarbital in the baboon, but does not substitute for pentobarbital in the pigeon or rhesus monkey nor for lorazepam in the baboon. Physical dependence liability has not been investigated in our study, but historical data suggest that it does substitute for barbiturates in dependent animals and can produce primary physical dependence. Self-administration studies in the rhesus monkey are in progress.

Methaqualone (CPDD 0007)

Methaqualone is active in the inverted screen test and in depression of locomotor activity. Drug discrimination studies reveal that it substitutes for pentobarbital in the rhesus monkey and diazepam in the rat, but not for pentobarbital in the pigeon or baboon nor for lorazepam in the baboon. In physical dependence studies, it substituted for pentobarbital in dependent rats, and signs of abstinence occurred upon withdrawal. Methaqualone was self-administered by rhesus monkeys. The potency of methaqualone was similar to that of pentobarbital in most studies.

Ethchlorovynol (CPDD 0015)

Ethchlorovynol was active in both the inverted screen test and suppression of locomotor activity. In drug discrimination studies it was identified as pentobarbital-like by both pigeons and rhesus monkeys, but has not yet been tested in benzodiazepine-trained animals. Studies on physical dependence liability in the rat are in progress. The compound is self-administered by rhesus monkeys. It appears to be approximately 1/2 to 3/4 as potent as pentobarbital.

Chloral Hydrate

In drug discrimination studies, chloral hydrate did not substitute for pentobarbital nor for lorazepam in the baboon. However, with intravenous self-injection in the baboon, reliable self-administration was maintained.

ABBREVIATIONS USED IN TABLES 1 - 9.

ED50 OR AD50: Antinociceptive assay (ED50, sc injection except where noted, mice) [Confidence limits are listed in the MCV and UM reports (Aceto et al. 1988; Woods et al. 1988)]: HP = hot plate; N = Nilsen; PPQ = phenylquinone; TF = tail flick; TFA = tail flick antagonism vs. morphine. These assays are performed at MCV, except for the HP and N (carried out at NIDDK, NIH).

I = inactive, without a reasonable dose-response relationship, or insufficiently active for statistical analysis.

EC50 Determinations:

These assays are done at UM. RBH = binding affinity, in the presence of 150mM NaCl, to rat cerebrum membrane preparations, in nM (parenthesized number is the +sodium/-sodium [+Na/-Na] ratio). EC50 was determined by displacement of 0.5nM [^{3}H]etorphine. The EC50 of morphine, for comparison = 23.6 (1.69). NE = no effect. NOTE: The present EC50 data cannot be directly compared with those from previous reports (Jacobson 1984, and preceding years) in which -Na values were quoted. However, the former numbers can be recalculated for comparison with those which are currently utilized through the use of the +Na/-Na ratio.

VD = electrically stimulated mouse vas deferens EC50 values, rounded to one significant figure. Agonist activity is stated using "E" followed by a negative number: E = 10^{-x} M, where x = the negative number, thus: 1E-3 = 0.001 M (1 mM), 1E-6 = 1 uM, and 1E-9 = 1 nM (parenthesized numbers are maximum percent inhibition at EC50); [bracketed letters: A = antagonized by 10^{-7} M naltrexone; NA = not antagonized by naltrexone; NE = no effect on inhibition of twitch; SA = slight antagonism by naltrexone]. Compounds which suppress the twitch and are not antagonized by naltrexone or UM 979 [NIH 8859, (-)-5,9-alpha dimethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-benzomorphan] are said to be non-opioid agonists (e.g. clonidine can suppress the twitch, but is not antagonized by naltrexone. It is a non-opioid agonist). (The effect of UM 979 is not noted in this report, but see the UM report (Woods et al. 1988) for these data). Compounds which bind with reasonable affinity in the rat brain homogenate assay, suppress the twitch in the VD, but are not blocked by narcotic antagonists may have antagonist properties, also. This is experimentally determinable by observing their antagonism to morphine's suppression of the twitch in the VD preparation (for these data see Woods et al. 1988).

Data From Monkey Colonies:
These data are from either MCV or UM. SDS = single dose suppression: NS = no suppression; CS = complete suppression; PS = partial suppression. (Parenthesized numbers = dose range studied, in mg/kg; if CS, then dose at which CS was observed is noted in the parentheses). Potency comparison with morphine [M] may be stated, in brackets.

NW = studies in non-withdrawn monkeys: PW = precipitated withdrawal at dose levels, in mg/kg, indicated in parentheses &/or comparison with naloxone [N], in brackets; NP = no precipitation; SP = slight precipitation.

Other Studies (OTHER):
RI = rat continuous infusion (from MCV): SM = substitution for morphine procedure [NS = no substitution for morphine; CM = complete substitution; PS = partial substitution], and PPD = primary physical dependence.

ND = non-dependent monkeys: M-like = morphine-like effect.

PPD = primary physical dependence (in the rhesus monkey).

SA = self-administration (from UM): NE = no effect; High = codeine-like; IN = intermediate between saline and codeine; SE = slight effect.

DD = drug discrimination (from UM).

Previous Reports (PR):
Previous work on a compound is noted by year, the year listed in

the monograph title (e.g. Problems of Drug Dependence 1986). Note that the date of publication of the monograph generally occurs one year after the titled year of the monograph. The data which have been published in previous reports are shown by a "PR" in the appropriate column (e.g., a PR (1983) in the SDS column would indicate that the SDS work was cited in "Problems of Drug Dependence 1983", which was published in 1984).

NOTE: The numbers used in the tables may be rounded. For precise values, and details of the procedures, see the MCV and UM reports in these Proceedings (Aceto et al. 1988; Woods et al. 1988).

Abbreviations for structural formulae: CPM=cyclopropylmethyl; CBM=cyclobutylmethyl; Me=methyl; Et=ethyl; n-Pr=propyl; i-Pr-isopropyl; n-Bu=butyl.

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Archer, S., Michael, J., Michael, M., Simon, E.J., Abdelhamid, E.M.E., Nelson, W.L., and Koolpe, G.A. Chloracryloyl amides and alpha-methylenelactones from naltrexone, oxymorphone and fentanyl. Neuropeptides 5:395-398, 1985.

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Woods, J.H., Medzihradsky, F., Smith, C.B., Winger, G.D., and Gmerek,D.E. Evaluation of new compounds for opioid activity. In: Harris, L.S. ed. Problems of Drug Dependence: 1987. National Institute on Drug Abuse Research Monograph. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1988, in press.

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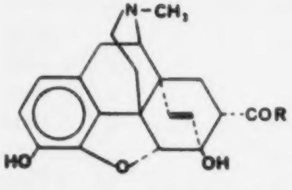
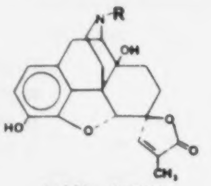
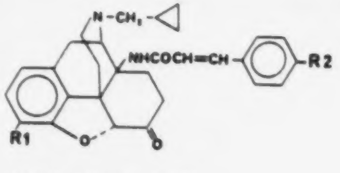
TABLE 1 - 4,5-EPOXYMORPHINANS[a]



9834: R=L-Phe-L-Leucinol



10322: R=CPM
10324: R=Me



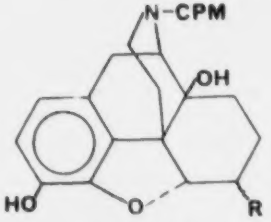
10420: R1=OMe, R2=Cl
10426: R1=OMe, R2=Br
10427: R1=OMe, R2=Me
10443: R1=OH, R2=Cl
10444: R1=OH, R2=Me
10445: R1=OH, R2=Br

	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES		OTHER STUDIES
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS	NW	
9834	5.0	-	-	-	0.3	4E-10(94)[A]	-	-	-
10322	-------PR (1986)-----				-	-	NS(0.1-1.0)[b]	PW(0.1-3.2)[b]	-
10324	-------PR (1986)-----				-	-	NS(1.0-32)[b]	-	-
10420	I	0.2	I	6.0	1.0	5E-9(95)[SA][b]	CS(0.05-0.8)[c]	PW(0.6-7.2)[d]	SA - NE[e]; DD -Codeine-like
10426	I	0.1	I	2.3	1.1	3E-9(54)[A][f]	CS(0.25-1.0)[c]	NP(0.1-4.0)	SA -High; DD -Codeine-like
10427	I	0.1	I	5.7	4.5	3E-8(62[A]	CS(0.25-4.0)	SP(1.0-10)[c]	SA -IN; DD -Codeine-like
10443	I	I	I	0.12[c]	-	-	NS(0.05)	PW(0.4)[g]	-
10444	I	I	I	0.2[c]	0.8	NE[h]	NS(0.05-0.2)[g]	-	-
10445	I	7.1	I	0.8[c]	0.8	NE[i]	NS(0.35-1.4)[g]	-	ND[j]; RI[k]

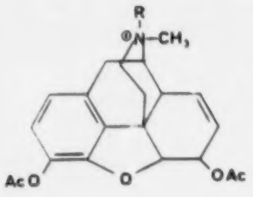
---

a) See text for explanation of column headings. b) Resembles irreversible agonists. Does not antagonize morphine. Effect partially on delta receptors. c) Long duration of action. d) Weak antagonist, but very long acting (3 days). e) Prolonged effect - slow return to codeine baseline. f) Antagonized morphine and U50,488. Also has kappa actions. g) Severe withdrawal, not relieved by morphine. h) Antagonized sufentanil. i) Antagonized sufentanil - "pure" antagonist. j) Pretreatment (0.35mg/kg) blocked acute effects of morphine for 2 weeks. k) Blocked weight loss associated with morphine.

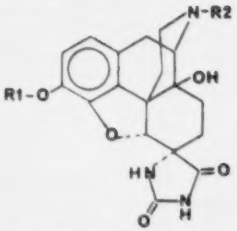
TABLE 2 - 4,5-EPOXYMORPHINANS (CONTINUED)[a]



10399: R=ALPHA OCOC(Me)=CH_2
10400: R=BETA OCOC(Me)=CH_2



10462: R=Me
10463: R=Et
10464: R=Pr
10465: R=ALLYL
10466: R=BENZYL



10516: R1=R2=Me
10519: R1=H, R2=CPM

	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES	
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS	NW
10399	I	I	I	0.008	0.63	1E-5(58)[NA]	NS(0.005-0.05)	PW(0.005-0.05)[5xN]
10400	I	I	I	0.02	10.5	1E-6(100)[SA]	NS(0.002-0.05)	PW(0.002-0.5)[1xN]
10462	I	I	I	I	>10uM	1E-7(32)[NA]	-	-
10463	I	I	I	I	>10uM	3E-8(48)[SA]	-	-
10464	I	I	I	I	>10uM	NE	-	-
10465	I	I	I	I	>10uM	NE	-	-
10466	I	I	I	I	>10uM	NE	-	-
10516	I	6.1	I	I	>6uM	NE	-	-
10519	-	I	I	I	55	3E-6(75)[A]	-	-

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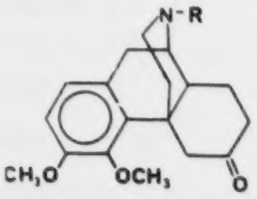
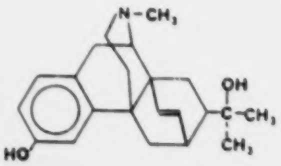
a) See text for explanation of column headings.

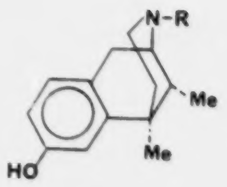
TABLE 3 - MORPHINANS AND 6,7-BENZOMORPHANS[a]



10017: R=CPM
10018: R=PHENETHYL



10500



7569: (-) R=Me
7571: (+) R=Me
10167: (+) $R=CH_2CH=CH-Cl$

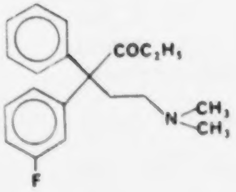
10502: $R=CH_2COOH$
10503: $R=CH_2COOEt$
10515: $R=(CH_2)_2COOEt$

	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES		OTHER STUDIES
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS	NW	
10017	PR[b]	3.7	3.9	I	-	-	PR-1982	-	-
10018	PR[b]	0.05	0.1	I	-	-	PR-1982	-	-
10500	I	0.2	I	I	-	-	-	-	-
7569	---PR (1986)-----				-	-	NS(1.0-4)--PR (1969)---		PR-RI(1986), PR-SA(1984)
7571	---PR (1986)-----				-	-	PR-1960, 1969	-	RI- SM-NS; PPD[c] PR[d]
10167	---PR (1983)-----				1.7	6E-7(93)[A]	-------------PR (1983)----------		-
10502	I	I	I	I	-	-	-	-	-
10503	I	I	I	I	-	-	-	-	-
10515	I	I	I	I	-	-	-	-	-

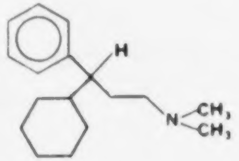
---

a) See text for explanation of column headings. b) 1982. c) Mild withdrawal (unlike morphine or (-)-metazocine. d) SA - 1984, ND - 1969.

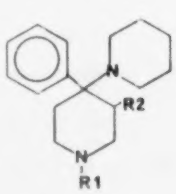
TABLE 4 - METHADONE-LIKE AND PETHIDINE-LIKE COMPOUNDS[a]



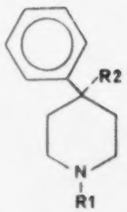
10261



10438



10012: R1=Me, R2=H
10013: R1=R2=Me
10014: R1=PHENETHYL, R2=H



10344: R1=PHENETHYL, R2=Me
10522: R1=Me, R2=COOEt
(PETHIDINE)

	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES		OTHER STUDIES
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS	NW	
10261	22.8	3.1	I	22.5	-	-	-	-	-
10438	I	I	I	I	-	-	-	-	-
10012	I	-	-	-	-	-	-	-	DD - I; PCP Receptor-I
10013	56	-	-	-	-	-	-	-	DD - I; PCP Receptor-I
10014	I	-	-	-	-	-	-	-	DD - I; PCP Receptor-I
10344	I	0.2	I	5.8	-	-	NS(0.03-2.0)	PW(0.03-4)[0.1xN]	-
(10522 05221)	4.8	0.8	7.8	I	-	-	CS(6.0)[PR-1955]	PR-1956	PR-DOGS(1955)

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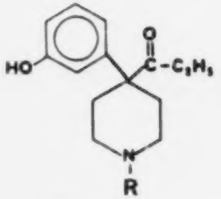
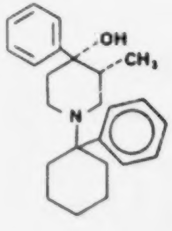
a) See text for explanation of column headings.

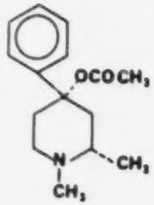
TABLE 5 - PETHIDINE-LIKE COMPOUNDS (CONTINUED)[a]



10453: $R=(CH_2)_3COOEt$
10454: $R=CH_2COOEt$
10467: $R=(CH_2)_2COOH$
10473: $R=CH_2COOH$
10475: $R=(CH_2)_3COOH$



10531



10542

MOUSE ED50 OR AD50				IN VITRO			MONKEY STUDIES		OTHER STUDIES
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS	NW	
10453	I	I	I	I	-	-	NS(2.5,10)	-	-
10454	I	I	I	I	-	-	NS(2.5,10)	-	-
10467	I	I	I	I	>10uM	NE	-	-	-
10473	I	I	I	I	>10uM	NE	-	-	-
10475	I	I	I	I	-	-	-	-	-
10531	-	1.2	15.3	I	68[b]	-	-	-	PCP Receptor= >1uM
10542	I	4.4	7.5	I	-	-	-	-	-

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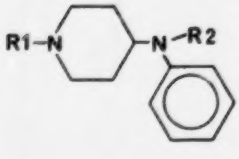
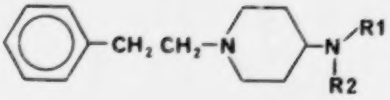
a) See text for explanation of column headings. b) Displacement assay using $[^3H]$DAGO, where morphine = 4 nM.

TABLE 6 - FENTANYL-LIKE COMPOUNDS[a]





10482: R1=BENZYL, R2=CO-n-Pr
10483: R1=BENZYL, R2=OCOEt
10485: R1=PHENETHYL, R2=COMe
10486: R1=PHENETHYL, R2=CO-n-Pr
10487: R1=PHENETHYL, R2=CO-i-Pr
10488: R1=PHENETHYL, R2=CO-n-Bu

10476: R1=H, R2=p-NITROPHENYL
10477: R1=p-METHOXYPHENYLACETYL, R2=PHENYL
10478: R1=COEt, R2=BENZYL

	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS
10482	I	0.4	11.5	I	472	1E-6(87[A]	CS(4.0)
10483	-	0.06	0.3	I	13	2E-7(100)[A]	CS(0.15,0.04)
10485	-	0.05	0.3	I	676	4E-7(100)[A]	CS(0.5)
10486	-	0.04	0.2	I	59	1E-7(72)[A]	CS(0.5)
10487	-	0.03	0.1	I	85	6E-8(99)[A]	CS(0.1)
10488	-	2.4	5.4	I	-	-	CS(5.0)
10476	I	0.9	15.5	I	-	-	-
10477	-	0.2	0.05	I	-	-	-
10478	-	0.2	1.7	I	-	-	-

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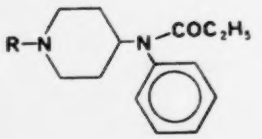
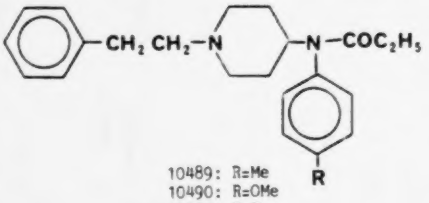
a) See text for explanation of column headings.

TABLE 7 - FENTANYL-LIKE COMPOUNDS (CONTINUED)[a]



10468: R=BENZYL
10484: R=PHENYLPROPYL
10493: R=THIENYLMETHYL
10505: R=THIENYLETHYL
10506: R=$CH_2CH(OH)$PHENYL
10538: R=$CH(Me)CH_2$THIENYL



10489: R=Me
10490: R=OMe
10491: R=F

	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS
10468 10492	I	6.9	I	I	2200 2310	NE NE	NS(3.0-18)
10484	-	0.09	0.85	I	78	1E-7(50)[A]	CS(1.0)
10493	I	I	I	I	2480	NE	NS(3.0-12)
10505	-	0.02	0.03	I	46	2E-8(98)[A]	CS(0.05)
10506	-	0.01	0.06	I	80	3E-8(100)[A]	CS(0.06)
10538	-	0.005	0.02	I	-	-	CS(0.03)
10489	-	0.3	0.9	I	85	4E-7(100)[A]	CS(1.0)
10490	-	0.1	0.5	I	91	2E-7(99)[A]	CS(0.5)
10491	-	0.01	0.07	I	16	2E-8(95)[A]	CS(0.08)

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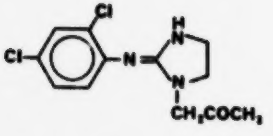
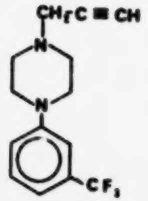
a) See text for explanation of column headings.

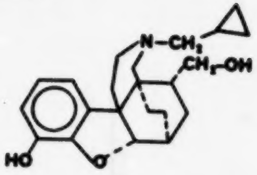
TABLE 8 - MISCELLANEOUS[a]



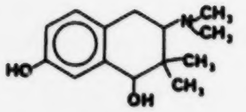
10318



10319



10412



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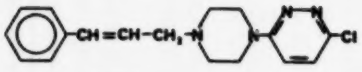

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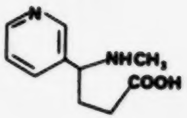

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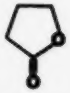

10540

	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES		OTHER STUDIES
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS	NW	
10318	-----PR (1985)------				--PR(1985)------		NE(1.0-32)[b][PR-1985]	-	-
(10319 10455)	-----PR (1986)------				--PR(1985)------		PR-1986	-	RI: SM-NS;PPD[c]
10412	I	0.03	I	2.7	0.55	1.5E-9(69)[A]	-	PW(0.004-0.1)[1xN]	-
10430	-	22	I	I	-	- -	-	-	
10496	I	3.1	I	I	1800	NE NS(2.5,10)	-	-	
10499	-	-	-	-	-	- NS(12,48)	-	-	
10540	-	7.9	I	-	-	- -	-	-	

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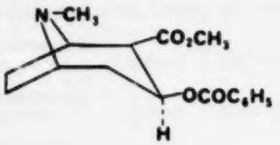
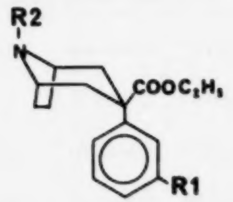
a) See text for explanation of column headings. b) Cumulative doses. c) Behavioral effects, no weight loss.

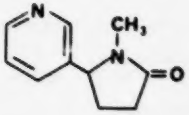
TABLE 9 - MISCELLANEOUS (CONTINUED)[a]



8211 (COCAINE)



10479: R1=OMe, R2=Me
10480: R1=OH, R2=Me
10481: R1=H, R2=Me
10529: R1=OH, R2=ALLYL
10530: R1=OH, R2=PHENETHYL



10498

	MOUSE ED50 OR AD50				IN VITRO		MONKEY STUDIES		OTHER STUDIES
NIH#	HP	PPQ	TF	TFA	RBH	VD	SDS	NW	
8211	-	2.8	I	I	-	-	-	-	PR-RI(1986)
10479	I	1.1	9.8	I	-	-	-	-	-
10480	I	4.5	I	I	-	-	-	-	-
10481	-	0.5	4.0	I	-	-	-	-	-
10529	-	7.7	I	I	-	-	-	-	-
10530	-	1.0	2.5	I	-	-	-	-	-
10498	-	I	I	17.3	-	-	NS(16,48)	-	-

---

a) See text for explanation of column headings.

TABLE 10. SUMMARY OF RESULTS FROM THE REPORT OF THE STIMULANT/DEPRESSANT TESTING GROUP OF THE COMMITTEE ON PROBLEMS OF DRUG DEPENDENCE

COMPOUND	INVERTED SCREEN IP	ACTIVITY CAGE IP	PHYSICAL DEPENDENCE		DRUG DISCRIMINATION				SELF-ADMINISTRATION
			SUBSTITUTION IP	PRIMARY IP	PB PIGEON IM	PB RHESUS PO	DZ RAT IP	BABOON PO	RHESUS IV
CPDD 0011 METHYLPENTYNOL	POSITIVE	POSITIVE	PARTIAL	POSITIVE	POSITIVE	POSITIVE	NOT TESTED	NOT TESTED	POSITIVE^{a}
CPDD 0011A METHYLPENTYNOL CARBAMATE	POSITIVE	NEGATIVE^{b}	PARTIAL	POSITIVE	IN PROG.	IN PROG.	NOT TESTED	NOT TESTED	NOT TESTED
CPDD 0012^{c} CHLORALADOL	POSITIVE TESTED IN SESAME OIL	POSITIVE	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED	NEGATIVE	NOT TESTED	NOT TESTED
CPDD 0013 CARBROMAL	POSITIVE	NEGATIVE^{d}	IN PROGRESS	IN PROGRESS	POSITIVE	PARTIAL	NOT TESTED	NOT TESTED	
CPDD 0014 CLOMETHIAZOLE EDISILATE	POSITIVE	POSITIVE	SLIGHT PARTIAL	NEGATIVE	POSITIVE	NEGATIVE^{e}	NEGATIVE	NEGATIVE-LZ	POSITIVE
CPDD 0016 TRICLOFOS	POSITIVE	POSITIVE	POSITIVE	PARTIAL-MILD WITHDRAWAL	IN PROG.	IN PROG.	POSITIVE	NEGATIVE-LZ NEGATIVE-PB^{f}	NOT TESTED
CPDD 0018 MEPROBAMATE	POSITIVE	POSITIVE	NOT TESTED^{g}	NOT TESTED^{g}	NEGATIVE	NEGATIVE	POSITIVE	POSITIVE-PB NEGATIVE-LZ	IN PROG.
CPDD 0007 METHAQUALONE	POSITIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE^{h}	POSITIVE	POSITIVE	NEGATIVE-LZ NEGATIVE-PB	POSITIVE
CPDD 0015 ETHCHLORVYNOL	POSITIVE	POSITIVE	IN PROG.	IN PROG.	POSITIVE	POSITIVE	NOT TESTED	NOT TESTED	POSITIVE
CHLORAL HYDRATE	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED	NOT TESTED	NEGATIVE-LZ^{i} NEGATIVE-PB	NOT TESTED

---

a) ONE MONKEY THAT TOOK THE GREATEST AMOUNT SHOWED WITHDRAWAL SIGNS. b) SLIGHT STIMULATION AT DOSES EFFECTIVE IN INVERTED SCREEN.
c) COMPOUND UNSTABLE IN WATER. d) RESULTS TO DATE INDICATE WILL AT LEAST BE PARTIAL BUT HIGHER DOSES NEED TO BE TESTED.
e) SIDE EFFECTS NOTED AT LOW DOSES BUT NOT AT HIGHER. f) HIGHEST DOSE WAS 56 MG/KG PO IN PENTOBARBITAL ANIMALS. HIGHER DOSES WERE NOT TESTED DUE TO LACK OF COMPOUND. g) HAS BEEN FOUND POSITIVE BY OTHER LABORATORIES. h) ONLY TESTED IN MIDAZOLAM TRAINED PIGEONS.
i) WITH IV SELF-INJECTION, RELIABLE SELF-ADMINISTRATION WAS MAINTAINED.

Dependence Studies of New Compounds in the Rhesus Monkey, Rat, and Mouse, 1987

M. Aceto, E. Bowman, L. Harris, and E. May

All the drugs except cocaine, (+)- and (-)-metazocine, γ-butyrolactone, cotinine and 4-(3-pyridyl)-4-methylaminobutyric acid were supplied by Dr. Arthur Jacobson, Medicinal Chemistry Section, NIADDK, NIH under the auspices of the Committee on Problems of Drug Dependence, Inc. The chemical structures of the test compounds were unknown to us when they were originally submitted.

For the most part, the procedures described by Seevers and his colleagues (1936, 1963) and Deneau (1956) regarding the facilities and training of the monkeys were used and a brief description follows. The monkeys were injected with 3.0 mg/kg s.c. of morphine sulfate every 6 hr for at least 90 days before being used. This dose schedule was reported by Seevers and Deneau (1963) to produce maximal physical dependence.

Modified procedures for the precipitated withdrawal (PPt-W) and single-dose suppression (SDS) tests were reported by Aceto and co-workers (1977 and 1978). The PPt-W test was initiated by the injection of a test drug 2 1/2 hr after an injection of morphine and the animals were observed for signs of withdrawal. The SDS test was started approximately 15 hr after the last dose of morphine at which time the animals were showing withdrawal signs. The onset and duration of action of the test drug were noted. In both tests, a vehicle control and an appropriate positive control (naloxone-hydrochloride, 0.05 mg/kg or morphine sulfate, 3.0 mg/kg) along with 2 or 3 different treatments (doses) of a test compound were randomly allocated to the 4 or 5 monkeys of a group. Usually, 3 or 4 groups per compound were used. All drugs were given subcutaneously (1 ml/kg) and the vehicle was water except where indicated. The observer was "blind" with regard to the treatment given. A minimal 2-week washout and recuperation period between tests was allowed. In the primary physical dependence (PPD) tests, the animals of a group received the drug every 4-6 hr for 30-50 days. They were placed in abrupt withdrawal and challenged with naloxone periodically, then observed for signs of physical dependence. All potency estimates are rough approximations only.

The rat-infusion method was reported by Teiger (1974) and certain modifications are indicated as follows. Semi-restrained, male, Sprague-Dawley rats were medicated by continuous infusion through indwelling intraperitoneal cannulas for 6 days with a drug. Rats were anesthetized after which each was fitted with a specially prepared cannula which was passed subcutaneously from the nape of the neck to the lateral side of the lower abdomen and then inserted into the peritoneal cavity. The cannula was anchored at both ends with silk sutures and attached to a flow-through, swivel mechanism which allowed the animal to move about in the cage and eat and drink normally. The swivel was connected to a syringe which was attached to a syringe pump. The animals received 7-10 ml of solution every 24 hr.

In the substitution for morphine (SM) test, the animals first received morphine (50 mg/kg/24 hr on the first day, 100 mg/kg/24 hr on the second day, and 200 mg/kg/24 hr from days 3-6). Then, a test drug was substituted for 2 days. The morphine controls received an infusion of water. The animals were observed for changes in body weight and for behavioral-withdrawal signs for 1/2 hr at 24, 48, 72 and/or 96 hr after stopping the infusion of morphine.

In the primary physical dependence (PPD) study, the rats received test compound for 6 days and then were placed in abrupt withdrawal and observed as above. Occasionally a drug was given with morphine.

Three mouse tests were used in our laboratory to provide a preliminary estimate of the potency and profile of activity of each test compound. The tests were the tail-flick agonist (TF) and the morphine antagonist (TV vs M) tests and the phenylquinone (PPQ) test (Dewey et al., 1970; Dewey and Harris, 1971). Reference-standard data for these tests are shown in Table 1. In addition, Dr. Jacobson sometimes provided us with estimated starting doses. These doses were based on results obtained from the mouse-hot plate (HP) (Eddy and Leimbach, 1953; Jacobson and May, 1965; Atwell and Jacobson, 1978) and Nilsen (N) (Perrine et al., 1972) tests from this laboratory. Reference data for these tests are shown in Table 2.

Table 1

Comparative Data-ED50, mg/kg s.c. (95% C.L.) of Selected Standards in 3 Mouse Agonist-Antagonist Tests

Drug	Tail-Flick Test	Tail-Flick Antagonist Test	Phenylquinone Test
Pentazocine	15% at 10.0	18 (12-26)	1.7 (1.0-2.5)
Cyclazocine	17% at 1.0[a]	0.03 (0.020-0.78)	0.01(0.005-0.03)
Nalorphine·HCl	None at 10.0	2.6 (0.7-10.0)	0.6 (0.03-1.44)
Naloxone·HCl	None at 10.0	0.04 (0.01-0.09)	No Activity
Naltrexone·HCl	None at 10.0	0.007 (.002-0.02)	No Activity
Morphine Sulfate	5.8(5.7-5.9)	-----	0.23(0.20-0.25)

[a] Mice were ataxic at 3.0 and 10.0 mg/kg but there was no further increase in reaction time.

Table 2

Comparative Data (ED50 mg/kg) [95% C.L.] from the Hot Plate and Nilsen Assays

	Hot Plate s.c./p.o.	Nilsen s.c./p.o.
Morphine Sulfate	0.98 (0.83-1.1) 6.3 (4.7-8.3)	1.3 (1.0-1.7) 8.3 (6.0-11.4)
Codeine Phosphate	6.8 (4.5-10.2) 13.5 (9.7-18.7)	7.4 (4.9-11.0) 14.7 (9.2-23.3)
Levorphanol Tartrate	0.2 (0.1-0.3) -	0.2 (0.16-0.3) 2.5 (1.7-3.7)
Meperidine·HCl	5.3 (4.0-7.1) -	- -
(-)-Metazocine·HBr	0.6 (0.5-0.9) 10.6 (8.0-14.1)	0.5 (0.3-0.7) 26.0 (21.0-33.0)
Dihydromorphinone·HCl	0.19 (0.15-0.25) 0.9 (0.7-1.2)	0.2 (0.15-0.3) 1.8 (1.5-2.1)
Nalorphine·HCl	9.9 (5.7-2.1) -	23.0 (16.2-32.7) -
Cyclazocine	1.5 (1.1-2.1)	0.1(0.07-0.16)
Pentazocine	9.3 (6.7-12.8) -	6.5 (4.4-8.8) -
Chlorpromazine·HCl	1.1 (0.9-1.5) -	- -

No dose response for naloxone and naltrexone.
Phenobarbital, amobarbital, oxazepam, flurazepam, meprobamate and mescaline are inactive on the hot plate test.

ACKNOWLEDGEMENTS

This study was supported by a contract (#271-85-8101) from the National Institute on Drug Abuse, Dr. Khursheed Asghar, Contract Officer. We also acknowledge the expert assistance of Susan M. Tucker and Ronald Jones. Special thanks to Dr. Billy R. Martin, Ramona Winckler and Laura Johnson for their help in the preparation of this manuscript using the Macintosh Plus computer.

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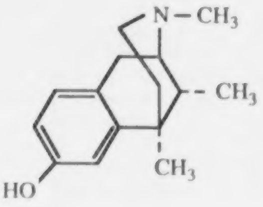
Tieger, D.G. Induction of physical dependence on morphine, codeine, and meperidine in the rat by continuous infusion, J Pharmacol Exp Ther, 190:408-415, 1974.

NIH 7569, (-)-2'-Hydroxy-2,5,9α-trimethyl-6,7-benzomorphan hydrochloride, (-)-Metazocine



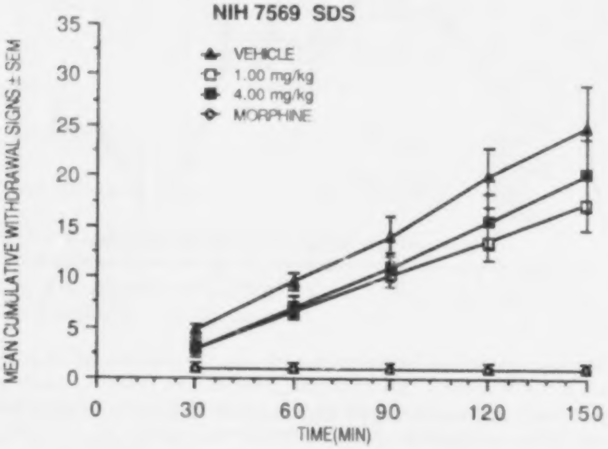
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - 0.81 (0.33-1.98)
2) TF vs. M - Inactive at 1.0 and 30.0
3) PPQ - 0.3 (0.1 - 0.9)
4) HP - 0.62 (0.45 - 0.85)
5) N - 0.46 (0.32 - 0.67)

MONKEY DATA

SDS

NIH 7659 did not substitute for morphine in the dose range of 1.0 - 4.0 mg/kg. There was a non-dose related reduction in withdrawal signs, designated wet-dog shakes and rigid abdominal muscles (see fig.). In addition, at the high dose, the signs salivation, drowsiness and slowing were noted.



NIH 7571, (+-Metazocine)

SEE OPTICAL ISOMER
NIH 7569 (ABOVE)

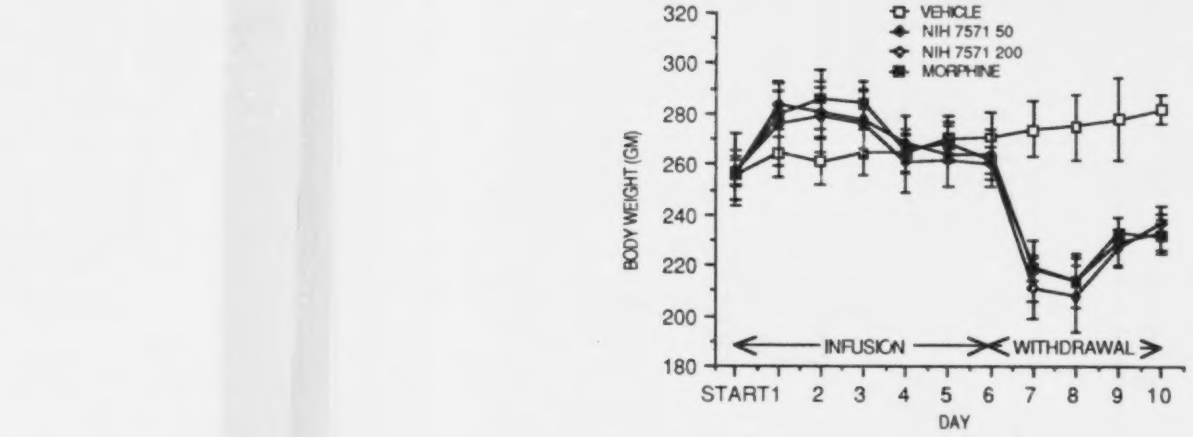
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - not done
3) PPQ - 14% at 3.0, 63% at 1.0 and 43% at 20.0
4) HP - Inactive at 20.0
5) N - Inactive at 20.0

RAT INFUSION

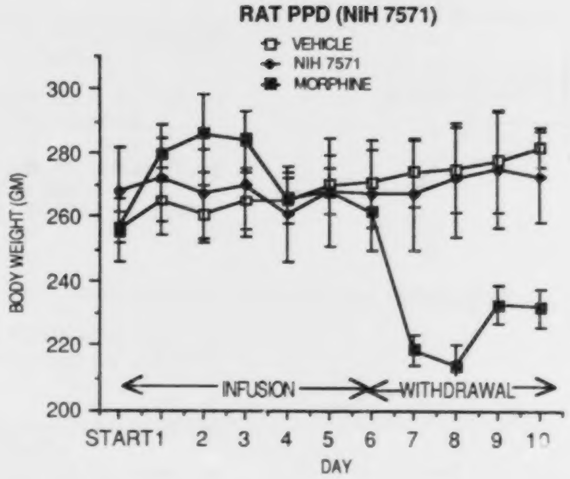
A. SM

As shown in the figure, substitution of H_20 or (+)- metazocine at 50 and 200.0 mg/kg/day for 2 days did not prevent loss of body weight or the emergence of withdrawal signs (table) in abruptly withdrawn morphine-dependent rats. (+)-Metazocine did not substitute for morphine.


RAT SM (NIH 7571)

B. PPD

When (+)-metazocine given for 6 days at a dose regimen 1/2 that of morphine, no loss of body weight was observed (see figure). However, 48 h after abrupt withdrawal, the rats showed certain overt signs designated (wet-dog shakes, rubbing and chewing but not squeaking and aggressiveness). Apparently, (+)-metazocine produces a mild withdrawal syndrome different from that produced by (-)-metazocine or morphine.

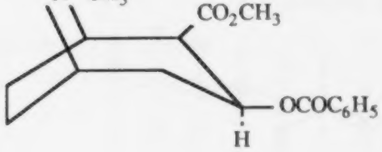


NIH 7571 Table
Substitution for morphine and primary physical dependence studies
in the rat with (+)-metazocine

Treatment	Hours after abrupt withdrawal and or substitution[a]			
	24	48	72	96
H_2O Controls[b] N=5	0.8	1.0	1.6	0
Morphine Infusion[c] N=5	12.2[d]	6.0[d]	5.0[d]	0.4
(+)-Metazocine Infusion[e,f] N=5	1.2	6.0[d,g]	4.6	0.2
Morphine[c] + (+)-Metazocine[h] N=4, 1 died on day 9	9.3[d]	4.0[d]	4.7	0.7
Morphine[c] + (+)-Metazocine[i] N=5, 1 died on day 9	6.9[d]	5.2[d]	0.6	1.5

[a]Mean number of withdrawal signs (hypersensitivity, squeaking, aggression, wet-dog shakes, rubbing and chewing) noted during 12 hr observation periods as specific intervals, [b]8 ml 24 hr, days 1-10, [c]50 mg/kg on day 1; 100 mg/kg on day 2; 200 mg/kg on days 3-6 and H_2O on days 7-10, [d]$p = 0.05$ or less (one-tailed Mann Whitney U-Test), [e]25 mg/kg on day 1; 50 mg/kg on day 2; 100 mg/kg on days 3-6 and H_2O on days 7-10, [f]all the rats in this groups showed swollen features in the head and neck region, [g]only signs noted were wet dog shakes, rubbing and chewing, [h]200 mg/kg on days 7-8, [i]50 mg/kg on days 7-8.

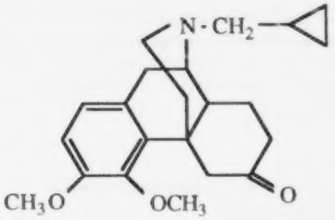
NIH 8211, (Cocaine·HCl)



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 1% at 1.0, 9% at 10.0 and 11% at 30.0
2) TF vs. M - 0% at 1.0, 10.0 and 30.0
3) PPQ - 2.83 (0.97 - 8.28)

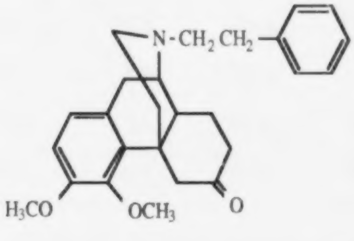
NIH 10017, (-)-N-Cyclopropylmethyl-3,4-dimethoxymorphinan-6-one hydrobromide



MOUSE DATA ED OR AD 50 95% C.L.) (mg/kg/s.c.)

1) TF -
2) TF vs. M - Inactive at 1.0 and 30.0
3) PPQ - 3.7 (1.6-8.8)
4) HP - 0.14 3.9 (2.8-5.5)
5) N - 11.1 (6.2-19.6)

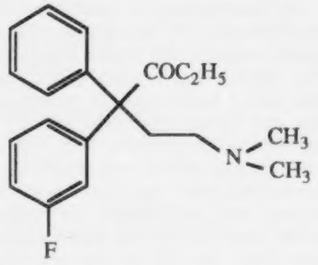
NIH 10018, (-)-3,4-Dimethoxy-N-(2-phenylethyl)morphinan-6-one hydrobromide



MOUSE DATA ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 0.1 (0.04-0.24)
2) TF vs. M - Inactive at 1.0 and 30.0
3) PPQ - 0.05 (0.02 - 0.12)
4) HP - 0.14 (0.01 - 0.18)
5) N - 0.02 (0.017 - 0.035)

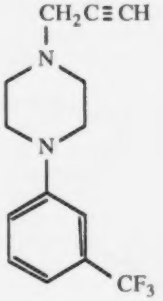
NIH 10261, *m*-Fluoronormethadone hydrochloride



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0, and 30.0
2) TF vs. M - 22.5 (11.9 - 42.4)
3) PPQ - 3.1 (1.4 - 6.8)
4) HP - 22.8 (20.5 - 25.2)

NIH 10319, 10455, N-Propargyl-N'-(3-trifluoromethylphenyl)piperazine hydrochloride



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 1.0, 10.0 and 30.0
3) PPQ - 1. 5.8 (1.5 - 22.0)
   2. 3.3 (0.34 - 32.54)
4) HP - 50% at 15% and 20.0, 60% at 100

Special TF vs M - (Naloxone Antagonism of ED80 of NIH 10319 in PPQ Test)

Naloxone Dose (mg/kg s.c.)	% Antagonism
40.0	11
20.0	14
10.0	25
1.0	11

RAT INFUSION

A. SM

As can be seen in the first figure and table, NIH 10319 did not substitute for morphine. Twenty-four hr after drug was substituted for morphine, the animals were vocalizing and aggressive but displayed few wet-dog shakes and little rubbing and chewing. However, severe tremors and eyelid ptosis, tail jerks associated with clonic convulsions, rigidity and arched backs were evident. This latter group of signs has never been seen in morphine-withdrawn rats. At 48 hours, vocalization and some aggression and a few wet-dog shakes were seen but the animals were still convulsing and dyspnea and ptosis were also observed. At 72 hr, additional morphine withdrawal signs emerged but the dominant feature was convulsions which developed when the animals attempted to move about. One animal was found dead. By 96 hr, 2 animals were showing more wet-dog shakes than all the morphine control animals. Another appeared moribund and died. At 120 hr, the two other animals still showed morphine-like withdrawal signs. On day 11, (data not shown) the survivors showed some weight gain. This observation may be related to the fact that the animals receiving NIH 10319 drank little H_2O during the substitution period compared with the morphine controls.

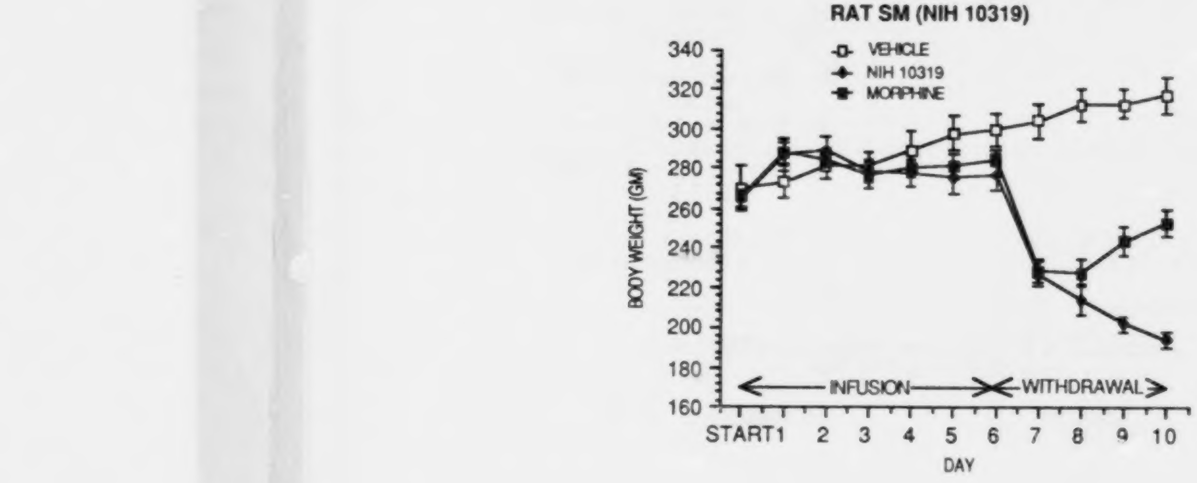


TABLE 1. Substitution of NIH 10319 for morphine in rat infusion test

	HR IN WITHDRAWAL[a]			
	24	48	72	96
H_2O Control (N=5)	0.6	0.4	0	0
Morphine Infusion H_2O Substitution (N=5)	9.2[b]	8.6	1.8	2.5[b]
Morphine Infusion NIH 10319 Substitution (N=5)	5.8	3.6	5.5	5.4

[a] Mean number of opioid-like withdrawal signs noted during 1/2 hr observation period, at specified intervals.

[b] Statistically significant differences ($p.<0.05$ or less)[b] and H_2O controls (8 ml/24 hr) or NIH 10319 substitution (200 mg/kg/24 hr, days 7-8). Withdrawal signs evaluated were hypersensitivity, squealing, aggression, wet-dog shakes, rubbing and chewing. Other overt signs observed are noted in the report. One-tail test (Mann-Whitney U-test).

B. PPD

In both groups of animals receiving different dose regimens of NIH 10319, some morphine-like behavioral withdrawal signs but no body weight loss were seen when drug was abruptly withdrawn (see Table 2). In addition, 2 animals showed protruding eyeballs and deep red pupils 24 hr after the drug was withdrawn. Regarding body weight, the animals gained during abrupt withdrawal (see fig. PPD-body weight). This was in sharp contrast with the weight loss observed in the NIH 10319- and morphine-treated rats in the substitution test. However, the animals lost weight during the infusion and this may be related to the sharp curtailment of water consumption (see fig. PPD-water consumption) especially during the first 24 hr and on days 4-6. On days 2 and 3, it seemed that some compensating mechanisms were triggered and the animals drank. During abrupt withdrawal of NIH 10319, water consumption increased.

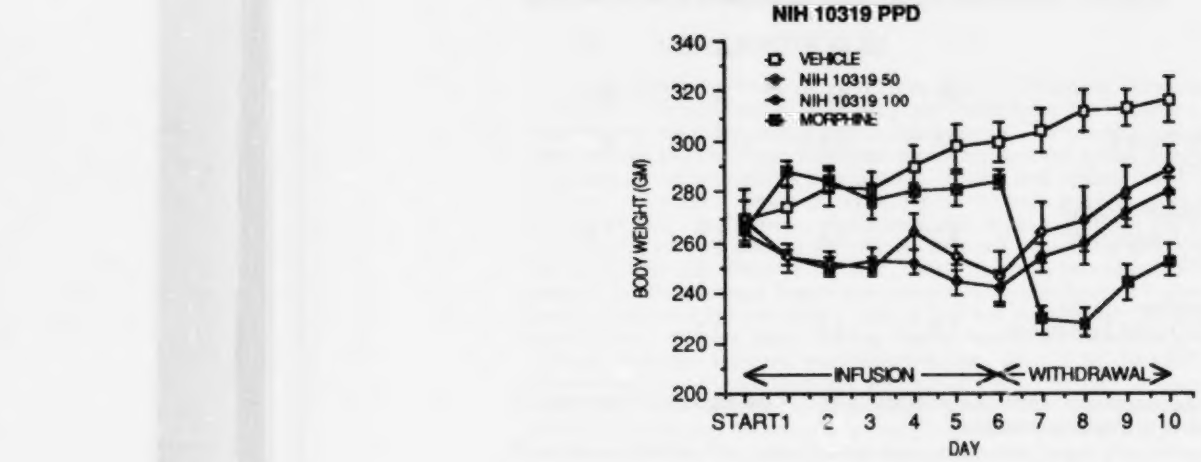
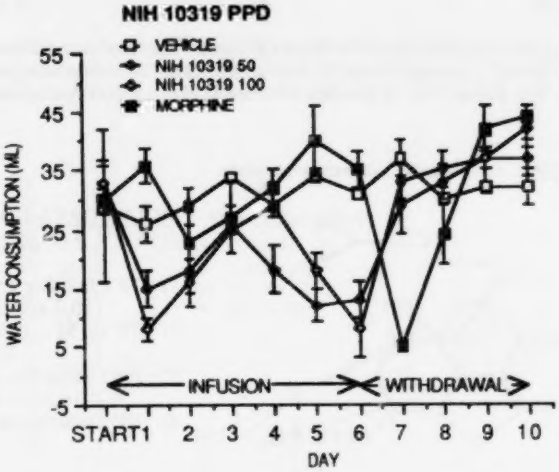


TABLE 2. Primary physical dependence rat infusion study (NIH 10319)

	HR IN WITHDRAWAL[a]			
	24	48	72	96
H_20 Control (N=5)	0.6	0.9	0	0
Morphine Infusion H_20 Substitution (N=5)	9.2[b]	8.6[b]	1.8	2.5
NIH 10319 Infusion[c] H_20 Substitution (N=5)	9.8[b]	1.0	1.3	1.3
NIH 10319 Infusion[d] H_20 Substitution (N=5)	5.8[b]	1.6[b]	2.4[b]	0.4[b]

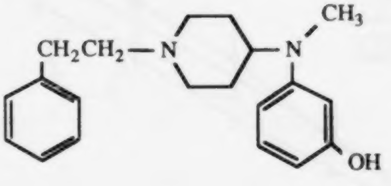
[a] Mean number of opioid-like withdrawal signs noted during 1/2 hr observation period, at specified intervals. Hypersensitivity, squealing, aggression, wet-dog shakes, rubbing and chewing. [b] Statistically significant differences (p 0.05 or less) between H_20 controls and NIH 10319c (100 mg/kg/24 hr, days 1-4; and 200 mg/kg/24 hr, days 5-6) or NIH 10319d (50 mg/kg/24, days 1-3; 100 mg/kg/24 hr, day 4 and 200 mg/kg/24 hr, days 5 and 6) or morphine controls. One-tailed test (Mann-Whitney U-test).



Conclusion

NIH 10319 appears to have unique properties. It produces some morphine-like behavioral withdrawal signs but no weight loss when given per se, yet, when given to abruptly withdrawn morphine addicts, it produces a different syndrome. One of the factors that can be associated with this curious profile of activity is the fact that H_20 consumption or regulation appears to be drastically affected.

NIH 10344, 4-(*m*-Hydroxyphenyl)methylamino-1-(2-phenylethyl)piperidine hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - 14% at .0, 13% at 10.0 and 8% at 30.0
2) TF vs. M - 5.8 (1.7 - 20.0)
3) PPQ - 0.2 (0.1 - 0.6)
4) HP - 0% at 20.0

MONKEY DATA

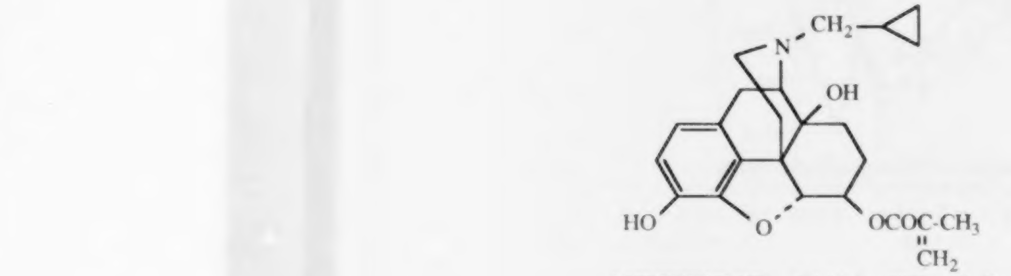
A. SDS

This drug did not substitute for morphine. At the highest dose, it appeared to exacerbate withdrawal.

B. PPt-W

The drug elicited dose-related withdrawal signs. The syndrome did not appear to be as severe as that produced by naloxone. Onset of action was prompt and duration was about 1 hr. A potency estimate is about 1/100 that of naloxone.

NIH 10399 , 6β-Naltrexol-α-methylacrylate



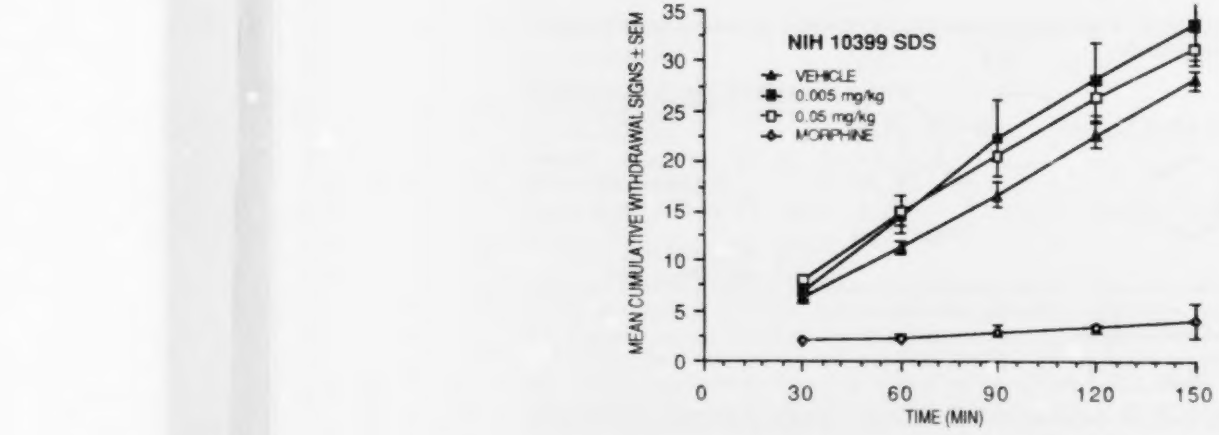
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 0.008 (0.004 - 0.02)
3) PPQ - Inactive at 1.0 and 10.0, 11% at 30.0
4) HP - Inactive at 20.0

MONKEY DATA (Vehicle - HCl + H_20)

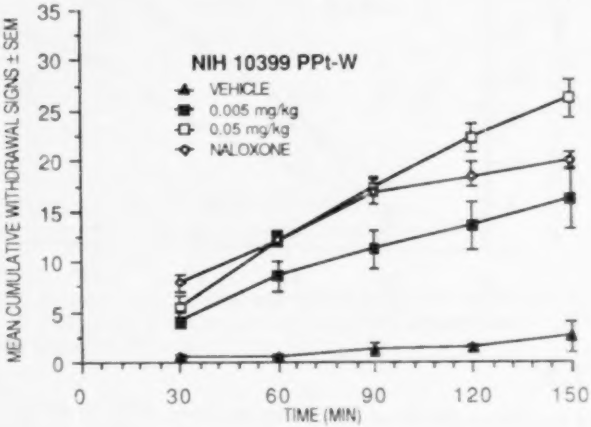
A. SDS

This drug did not substitute for morphine. Instead, it exacerbated withdrawal (see SDS fig.).

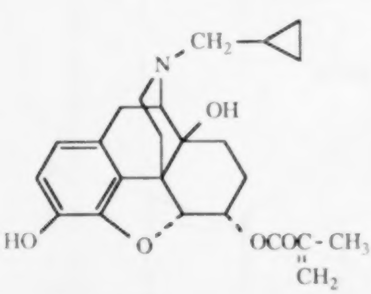


B. PPt-W

As shown in the figure, NIH 10399 precipitated withdrawal in nonwithdrawn addicts. Onset of action is rapid. The duration of action is longer than that of the reference standard naloxone. Potency is about 5 times that of naloxone.



NIH 10400, 6α-Naltrexol-α-methylacrylate



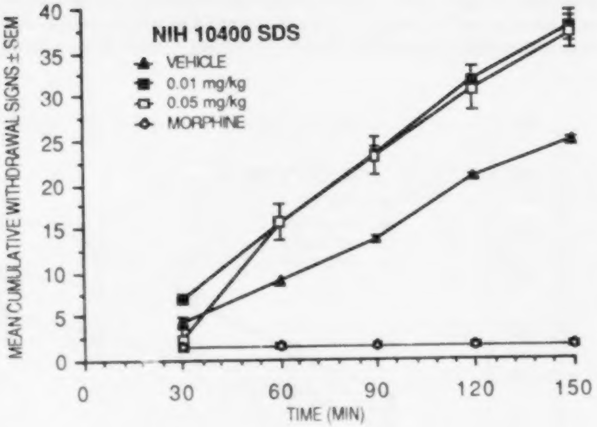
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 0.02 (0.009 - 0.07)
3) PPQ - Inactive at 1.0 and 10.0, 11% at 30.0
4) HP - Inactive

MONKEY DATA

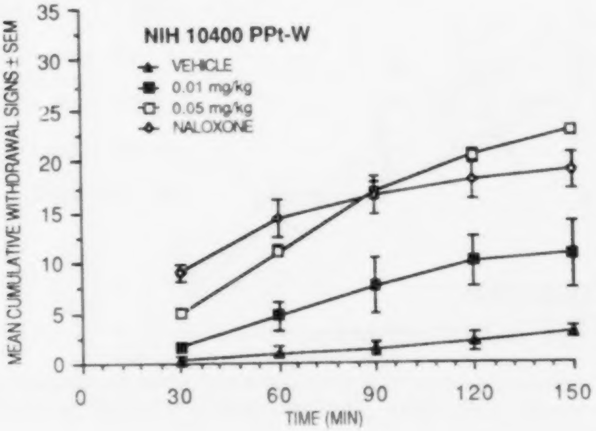
A. SDS (Vehicle - HCl and H_2O)

As shown in the figure, NIH 10400 did not substitute for morphine in the dose range 0.002 - 0.05 mg/kg. The drug exacerbated withdrawal at the 2 higher doses.

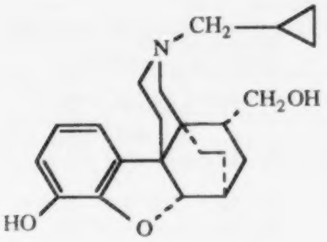


B. PPt-W

In nonwithdrawn morphine-dependent monkeys, NIH 10400 precipitated withdrawal. The drug had a slower onset and longer duration of action than naloxone and is approximately equipotent with it. The 0.002 mg/kg dose was not plotted because N=1.



NIH 10412. (±)-3-Cyclopropylmethyl-1,2,3,4,5,6,7,7a-octahydro-9-hydroxy-4aH-4a,7-ethanobenzofuro(3,2-e)isoquinoline-5-methanol



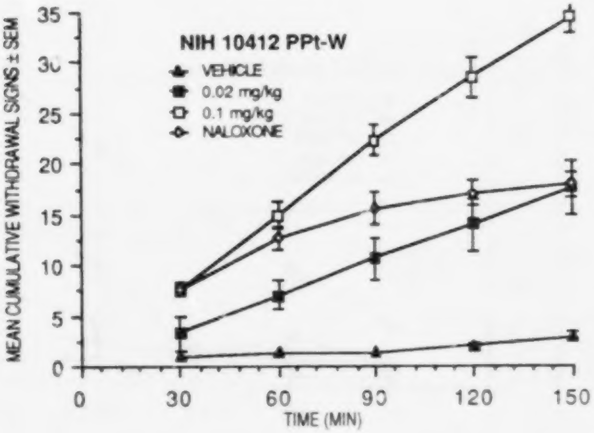
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - 3% at 0.001, 37% at 0.01, 35% at 0.1, 39% at 1.0, 41% at 10.0
2) TF vs. M - 2.7 (0.9-7.9)
3) PPQ - 0.03 (0.01 - 0.11)
4) HP - Inactive at 20.0

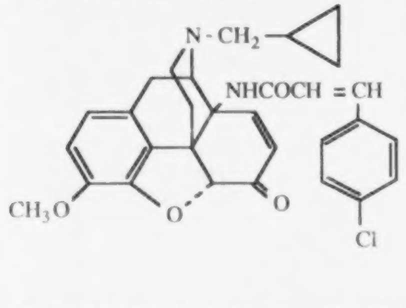
MONKEY DATA (Vehicle - lactic acid + H_2O)

PPt-W

NIH 10412 promptly precipitated withdrawal in a dose-related manner (see figure). The drug has a longer duration of action (longer than 2 1/2 hr) as opposed to naloxone (90 min).



NIH 10420. 14β-(*p*-Chlorocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

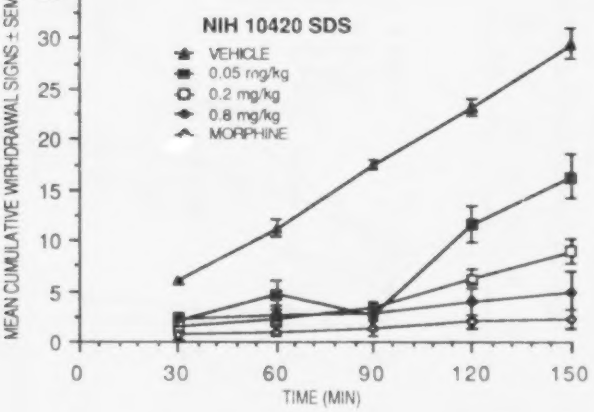
1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - 6.0 (2.1 - 17.4)
3) PPQ - 0.2 (0.05 - 0.6)[a]
4) HP - Inactive at 20.0

[a]Vehicle - Tween 80 + H_2O.

MONKEY DATA (Vehicle Tween 80 + H_2O)

A. SDS

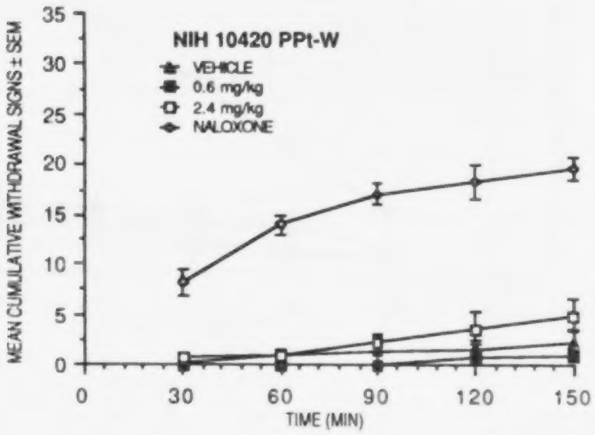
As shown in the fig., NIH 10420 produced a dose-related attenuation of withdrawal signs. The onset of action was delayed by 30 min but the duration was at least 2 1/2 hr.



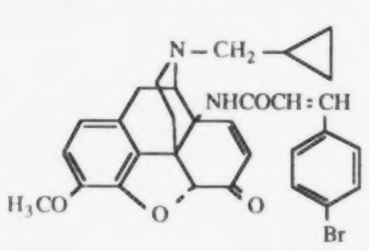
B. PPt-W

During the period when naloxone was active, NIH 10420 showed very weak antagonist properties (see figure). However, after 3-4 hr, the animals showed signs of withdrawal which persisted for a long period in spite of the fact that morphine was given. These withdrawal signs were: fighting, contact avoidance, slowing, wet-dog shakes, vocalization when abdomens palpated even though their abdominal muscles were relaxed. The animals were in obvious distress when handled. Two monkeys were in this state of withdrawal for 3 days even though morphine was given.

NIH 10420 is an unusual compound. It nearly substituted for morphine in withdrawn addicts but produced a delayed but long term withdrawal state when given to nonwithdrawn addicts. This drug action in monkeys is reminiscent of that of buprenorphine except that buprenorphine would precipitate a full withdrawal syndrome without delay.



NIH 10426. 14β-(*p*-Bromocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

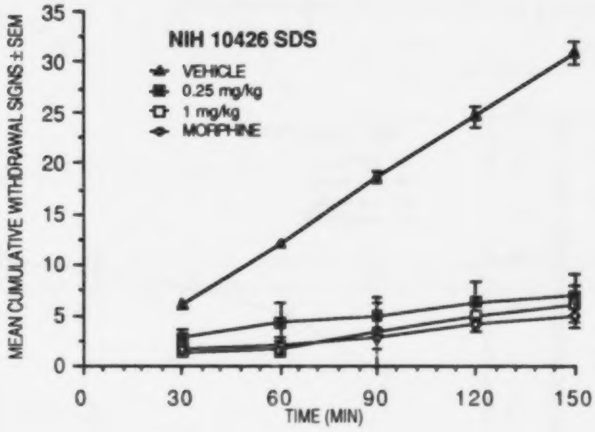
1) TF - 7% at 0.1, 45% at 1.0, 25% at 10.0 and 19% at 30.0
2) TF vs. M - 2.3 (0.6 - 8.5)
3) PPQ -0.11 (0.03 - 0.4)
4) HP - Inactive at 5.0 to 20.0

Vehicle - Tween 80 + H_2O.

MONKEY DATA

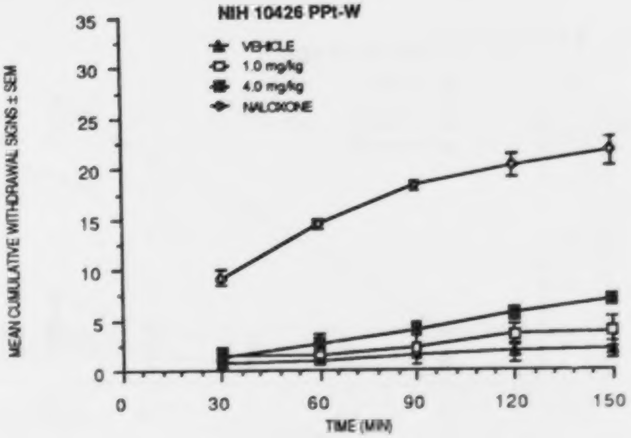
A. SDS

NIH 10426 nearly completely substituted for morphine. The drug acted promptly and had a long duration of action. In fact, most of the animals did not require morphine at noon and 2 of the monkeys at the highest dose did not require it until the 6 p.m. injection period.

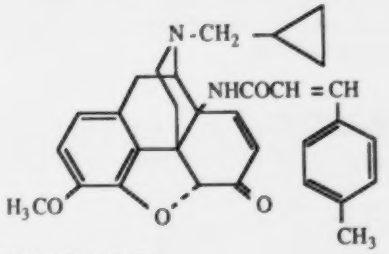


B. PPt-W

This compound did not precipitate a withdrawal syndrome in the dose range tested (see fig.). At the highest dose, the only signs noted during the entire 2 1/2 hr observation period were fighting, contact avoidance, restlessness, drowsiness, tremors, wet-dog shakes and masturbation. The 0.1 mg/kg dose was not plotted because N<3.



NIH 10427. 14β-(*p*-Methylcinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate



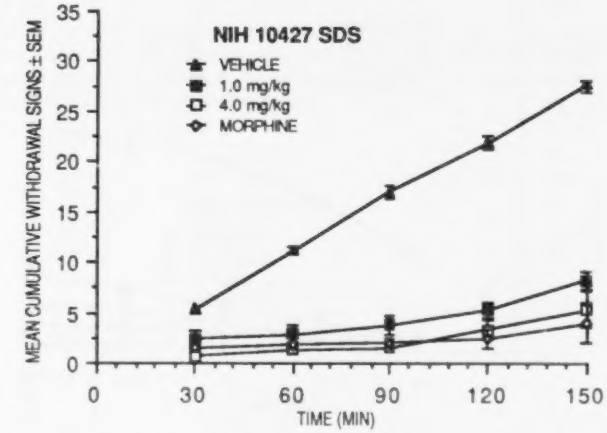
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - 19% at 1.0, 21% at 10.0 and 46% at 30.0
2) TF vs. M - 5.7 (2.4 - 13.7)
3) PPQ - 0.1 (0.01 - 1.3)
4) HP - Inactive at 20.0

MONKEY DATA

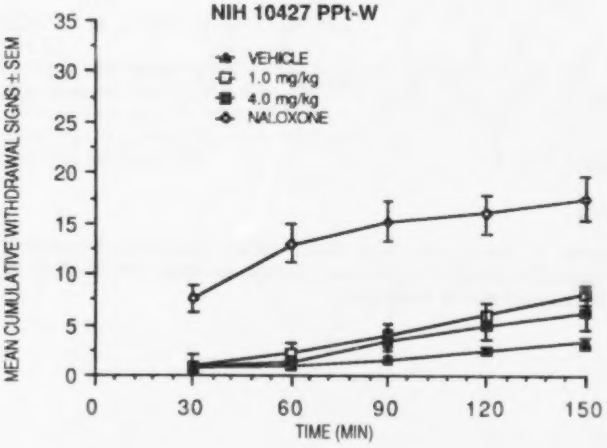
A. SDS

As shown in the figure, NIH 10427 nearly substituted completely for morphine. The onset of action was prompt; however, the duration of action was shorter than that of morphine.

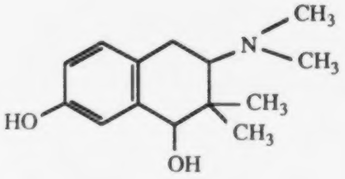


B. PPt-W

Although NIH 10427 showed only weak actions regarding precipitated withdrawal during the usual 150 min of the assay (see fig.), some of the animals showed signs of withdrawal through the next day. In the monkey receiving the highest dose, morphine at twice the normal dose did not relieve withdrawal. The 10.0 mg/kg dose was not plotted.



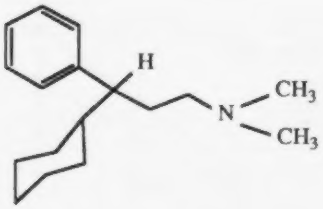
NIH 10430, 2,2-Dimethyl-3-(dimethylamino)-7-hydroxy-1-tetralol



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 6% at 1.0, 10% at 3.0, 37% at 10.0 and 43% at 30.0
3) PPQ - 21.9 (6.8 - 70.8)

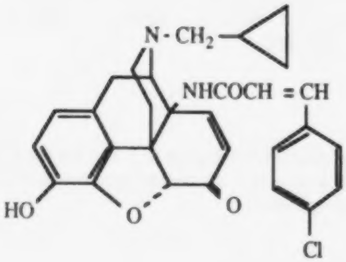
NIH 10438, 3-Cyclohexyl-1-N,N-dimethylamino-3-phenylpropane hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 7% at 1.0, 23% at 10.0, and 34% at 30.0
4) HP - Inactive at 5.0 and 20.0

NIH 10443, 14β-(*p*-Chlorocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnormorphinone mesylate



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 0.12 (0.07 - 0.23)
3) PPQ - 23% at 3, 34% at 10.0, 69% at 30.0 and 54% at 60.0
4) HP - Inactive at 20.0

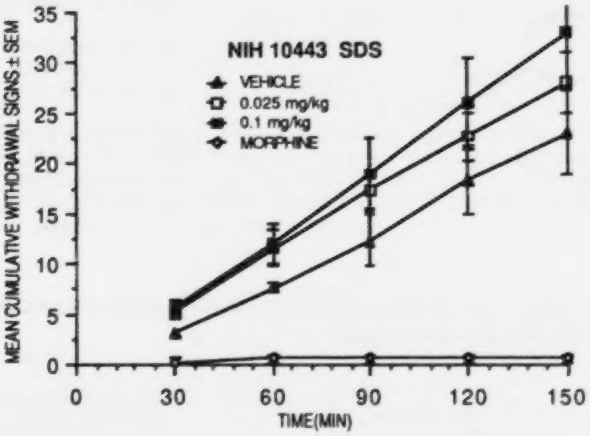
TF vs M - Special Time Course (ED50 of NIH 10443 vs ED80 of Morphine)

Pretreatment Time (hr)	% Antagonism
2	73
24	86

MONKEY DATA

A. SDS

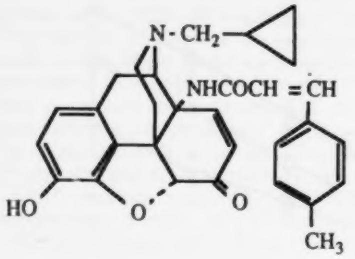
Withdrawal was exacerbated and very frequent masturbation was seen. Morphine promptly attenuated the withdrawal syndrome (see graph).



B. PRELIM. PPt-W

Another monkey received a cumulative dose of 0.4 mg/kg in 1 hr. By the third 15 min interval, the animal developed a severe withdrawal syndrome which was not relieved by a double dose of morphine.

NIH 10444, 14β-(*p*-Methylcinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnormorphinone mesylate



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

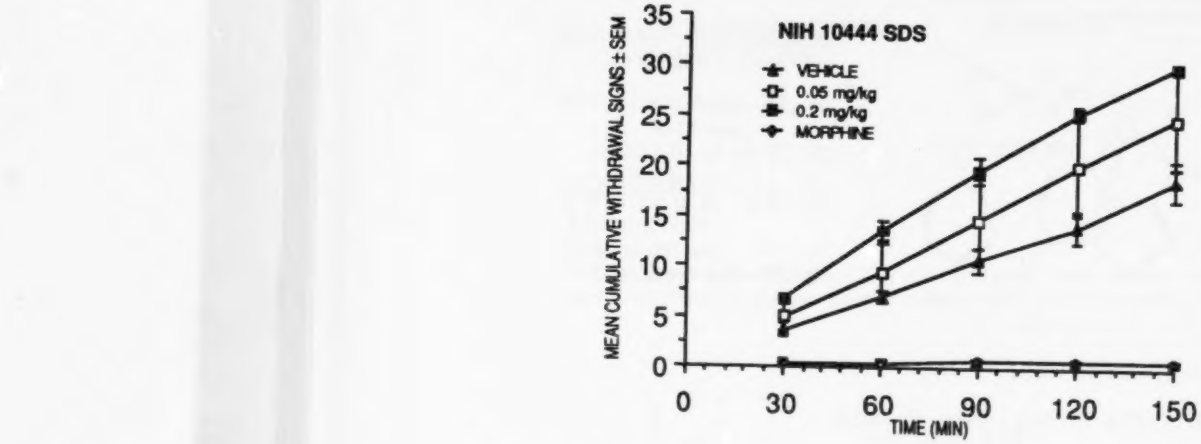
1) TF - Inactive at 1.0, 10.0 and 30.0[a]
2) TF vs. M - 0.2 (0.09 - 0.6)[a,b]
3) PPQ - 3% at 0.5, 19% at 1.0, 17% at 10.0 and 0% at 30.0[a]
4) HP - Inactive at 20.0

[a] Vehicle - Tween 80 + H_2O.

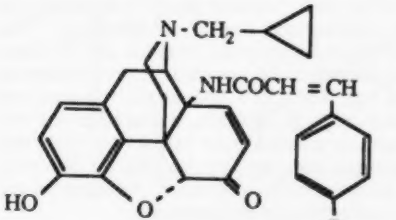
MONKEY DATA (Vehicle - Tween 80 + H_2O)

SDS

As shown in the fig., NIH 10444, exacerbated withdrawal. At the highest dose, severe tremors and retching were noted in one animal. Morphine was given 30 min after drug to terminate withdrawal. However, this animal was still retching and/or vomiting 1 1/2 hr later. Another monkey receiving the highest dose also showed severe retching and was given morphine. This monkey stopped retching and had relaxed abdominal muscles 30 min later. The third monkey receiving the highest dose did not show severe tremors or retching. However, this animal still had rigid abdominal muscles and vocalized when palpated 24 hr after drug. In addition, it moved about very slowly. At the low dose, all the animals showed more withdrawal signs than vehicle-treated animals but responded promptly to morphine. The drug appears to be either a long acting or irreversible antagonist. Needless to say, in this dose range NIH 10444 does not substitute for morphine.



NIH 10445. 14β-(*p*-Bromocinnamoylamino)-7,8-dihydrocyclopropylmethylnormorphinone mesylate



MOUSE DATA ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 0.8 (0.6 - 1.0)
3) PPQ - 7.1 (3.1 - 16.4)
4) HP - Inactive at 20.0

TF vs M - Special Time Course (ED50 of NIH 10443 vs ED80 of Morphine)

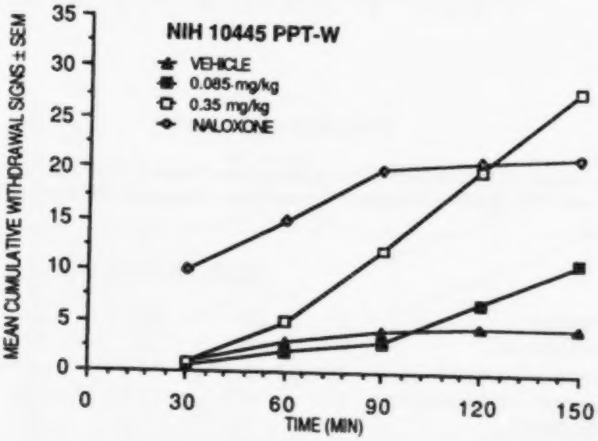
Pretreatment Time (hr)	% Antagonism
24	93
48	39
72	45

MONKEY DATA (Vehicle lactic acid + H_2O)

A. PPt-W

After a 1-hr delay, NIH 10445 precipitated withdrawal. However, the data shown in the figure do not begin to express the results because it illustrates only the first 150 min of the experiment. The drug had a long duration of action and its action could not be terminated with multiple double injections of morphine. One animal receiving the low dose remained in withdrawal for 4 days in spite of the extra doses of morphine. Another monkey receiving a dose of 0.085 mg/kg also remained on withdrawal despite efforts to reverse the syndrome with morphine. This latter animal was finally anesthetized. Recovery was slow. The monkey receiving the highest dose died after 4 days. Of the 7 monkeys receiving NIH 10445 (including 1 tested in the preliminary experiment) 3 died, and 2 showed attenuated abrupt and precipitated withdrawal syndromes during a 4-6 week period after receiving drug.

In another study involving a non-addicted monkey, pretreatment with NIH 10445 (0.35 mg/kg) blocked the acute effects of morphine for 2 weeks. Obviously, NIH 10445 is an unusual drug that merits much more study.



B. RAT INFUSION

Special Combination Study

As shown in the fig., at a dose of 10 mg/kg/day in combination with morphine, NIH 10445 nearly blocked the weight loss commonly associated with morphine withdrawal in addicted rats. In addition, per se the drug did not significantly affect body weight. No overt withdrawal in addicted rats. In addition, per se the drug did not significantly affect body weight. No overt withdrawal signs were seen during abrupt withdrawal either in combination with morphine or by itself (see table).

COMBINATION STUDY (NIH10445)

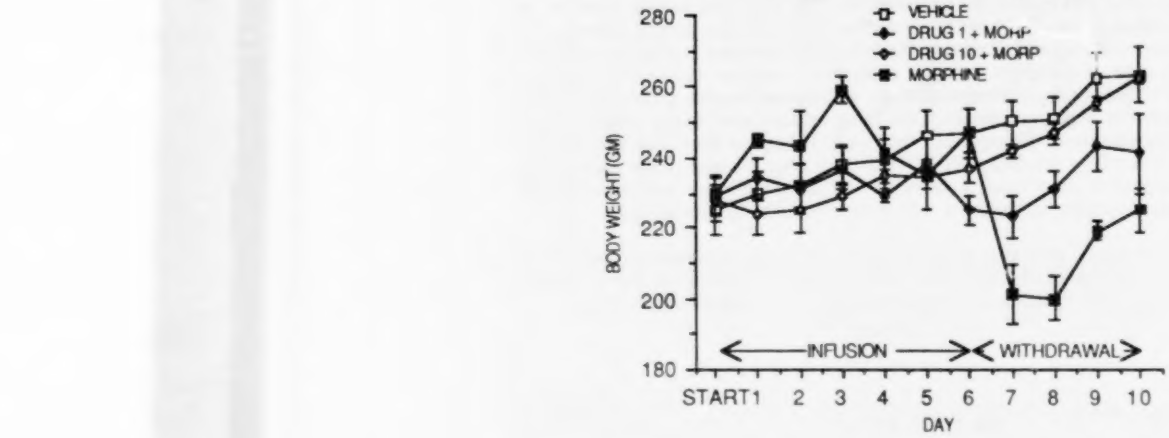


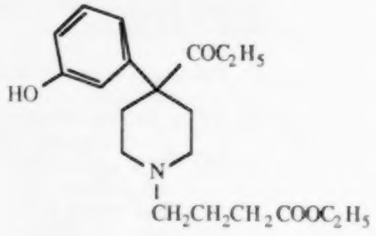
TABLE (NIH 10445)

Means of Withdrawal Signs of Morphine-Treated, NIH 10445-Treated and NIH 10445 + Morphine-Treated Rats Compared With H_2O Controls

	HR IN WITHDRAWAL[a]				
Treatment	0	24	48	72	96
H_2O Controls[c]	0.5	0	0	0.5	0
Morphine Controls[d]	1.2	5.3[b]	13.3[b]	13.7[b]	14.01[b]
Morphine + NIH 10445[e]	0.3	3.3	2.3	2.3	2.0
NIH 10445[f]	1.0	3.0	0.3	1.0	0.3

[a]Mean number of withdrawal signs, [b]Significant at $p< 0.05$. One-tailed test (Mann-Whitney U-test).,Hypersensitivity, squeaking, aggression, wet-dog shakes, rubbing and chewing,[c] N=4, 7ml/24 hr. Note - H_2O controls are not vehicle controls, [d] N=3, 50 mg/kg 1st day, 100 mg/kg 2nd day, 200 mg/kg days 3-6; H_2O days 7-10, [e] N=3, morphine as above + 10 mg/kg NIH 10445 (suspended in Tween 80 + H_2O,[f] N=3, NIH 10445 = 10 mg/kg/24 hr (suspended in Tween 80 + H_2O).

NIH 10453. N-(3-Carbethoxypropyl)-N-norketobemidone oxalate



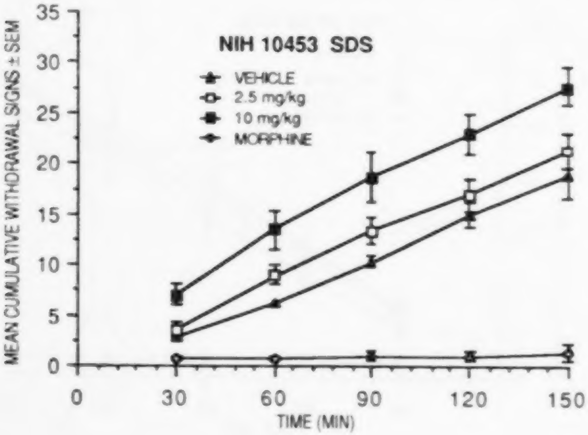
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 11% at 1.0, 2% at 10.0 and 0% at 30.0
3) PPQ - Inactive at 1.0, 10.0 and 30.0
4) HP - Inactive at 5.0 and 20.0

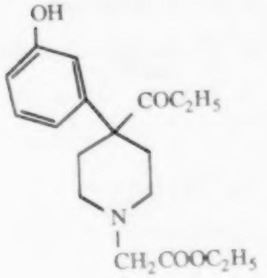
MONKEY DATA

SDS

NIH 10453 did not substitute for morphine at doses of 2.5 and 10.0 mg/kg. However, the drug may have exacerbated withdrawal (see graph).



NIH 10454. N-Carbethoxymethyl)-N-norketobemidone oxalate



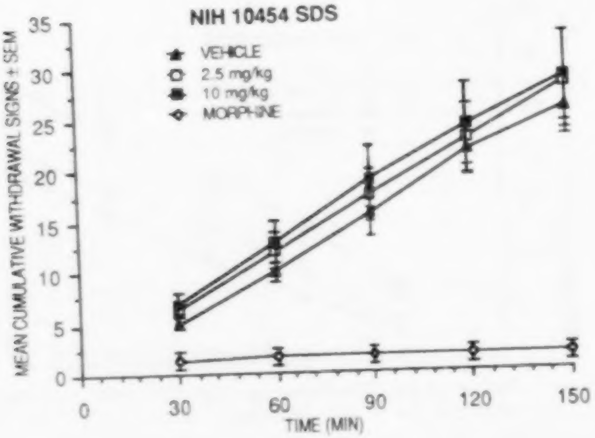
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 17% at 0.1, 20% at 1.0, 43% at 10.0 and 46% at 30.0
4) HP - Inactive at 5.0 and 20.0

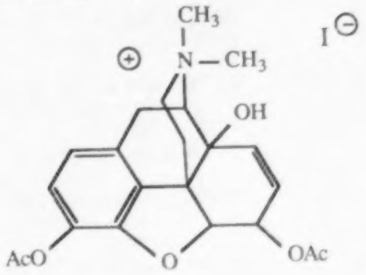
MONKEY DATA

SDS

As shown in the fig., NIH 10454 did not substitute for morphine at doses of 2.5 and 10.0 mg/kg. One monkey receiving the highest dose retched more frequently than controls.



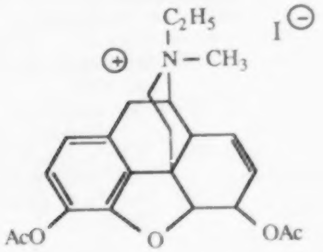
NIH 10462. Heroin methiodide



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 6% at 1.0, 11% at 10.0 and 19% at 30.0
4) HP - Inactive at 5.0 and 20.0

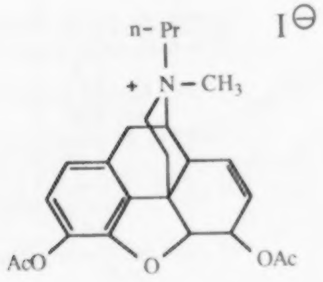
NIH 10463. Heroin ethiodide



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - Inactive at 1.0, 10.0 and 30.0
4) HP - Inactive at 5.0 and 20.0

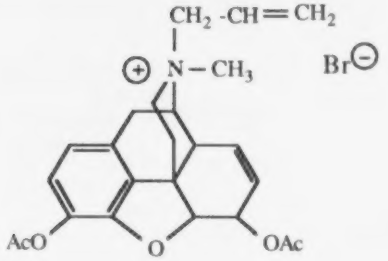
NIH 10464. Heroin propiodide



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 14% at 1.0, 6% at 10.0 and 13% at 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 13% at 0.1, 45% at 1.0, 39% at 10.0 and 60.0 at 30.0
4) HP - Inactive at 5.0 and 20.0

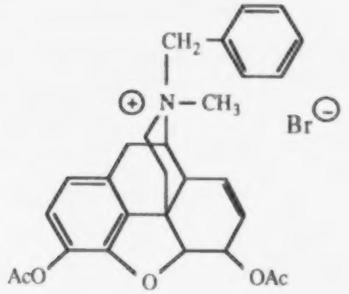
NIH 10465. Heroin allobromide



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - 1% at 1.0, 9% at 10.0 and 12% at 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 9% at 1.0, 14% at 10.0 and 26% at 30.0
4) HP - Inactive at 5.0 and 20.0

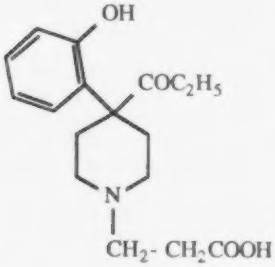
NIH 10466. Heroin benzobromide



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 20% at 1.0, 31% at 10.0 and 4% at 30.0
4) HP - Inactive at 5.0 and 20.0

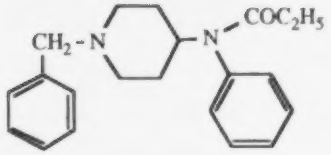
NIH 10467. N-(2-Carboxyethyl)-N-norketobemidone hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 9% at 1.0, 17% at 10.0 and 37% at 30.0
4) HP - Inactive at 5.0 and 20.0

NIH 10468 (or 10492). N-(1-Benzyl-4-piperidyl)-N-phenylpropanamide hydrochloride



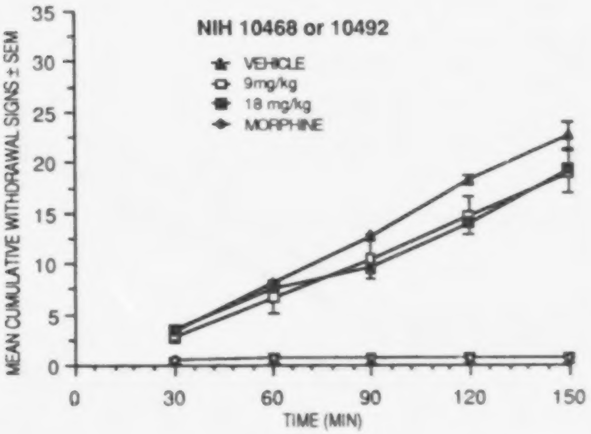
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 6.9 (2.9 - 16.7)
4) HP - Inactive at 5.0 and 20.0

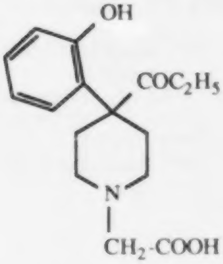
MONKEY DATA

SDS

NIH 10468 did not substitute for morphine at doses of 0.3 - 18.0 mg/kg. At the highest dose, one animal convulsed and was given pentobarbital. In addition, all showed severe tremors at this dose. The 0.3 and 3.0 mg/kg doses were not plotted because N < 3 (see fig.).



NIH 10473. N-Carboxymethyl-N-norketobemidone hydrobromide



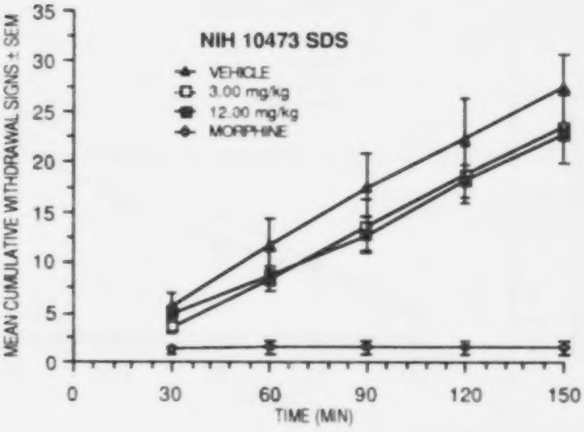
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - Inactive at 1.0, 10.0 and 30.0
4) HP - Inactive at 5.0 and 20.0

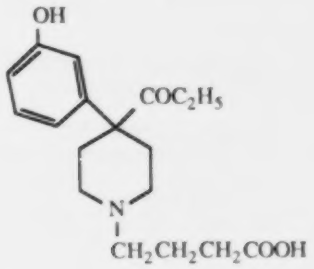
MONKEY DATA

SDS

At doses of 3.0 and 12.0 mg/kg, NIH 10473 neither substituted for morphine nor exacerbated withdrawal (see fig.).



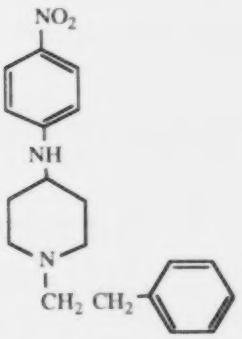
NIH 10475. N-(3-Carboxypropyl)-N-norketobemidone



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 6% at 1.0, 20% at 10.0 and 40% at 30.0
4) HP - Inactive at 5.0 and 20.0

NIH 10476. 1-Phenethyl-4-(4-nitrophenyl)aminopiperidine dihydrochloride

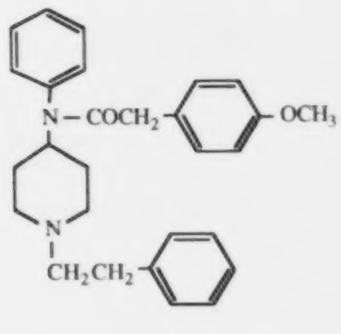


MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 5.7 (2.98 - 10.91)[a]
2) TF vs. M -
3) PPQ -
4) HP - Inactive at 20.0

[a] Drug supply exhausted

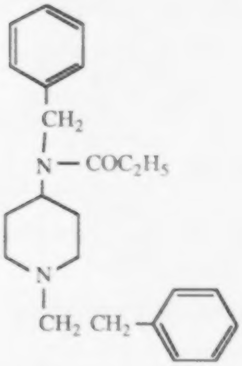
NIH 10477. 1-Phenethyl-4-(N-phenyl-p-methoxyphenylacetyl)aminopiperidine hydrochloride



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 0.05 (0.02 - 0.7)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.2 (0.07 - 0.4)

NIH 10478. 1-Phenethyl-4-(N-benzyl,N-propionyl)aminopiperidine hydrochloride

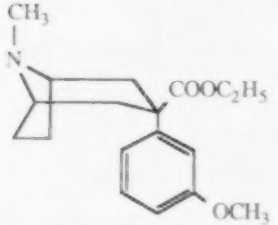


MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 1.7 (0.9 - 3.0)
2) TF vs. M - Inactive at 1.0,

   10.0 and 30.0
3) PPQ - 0.2 (0.1 - 0.4)

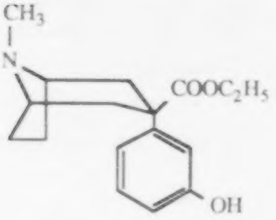
NIH 10479. Ethyl 3α-(3-methoxyphenyl)-N-methyl-8-azabicyclo[3.2.1]-octane carboxylate hydrochloride



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 9.8 (6.0 - 16.0)
2) TF vs. M - Inactive at 1.0,
   10.0 and 30.0
3) PPQ - 1.1 (0.2 - 5.1)
4) HP - Inactive at 5.0, 50% at
   20.0

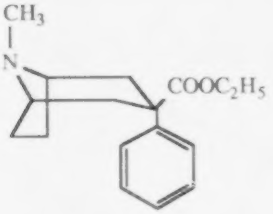
NIH 10480. Ethyl 3α-(3-hydroxyphenyl)-N-methyl-8-azabicyclo[3.2.1]-octane carboxylate hydrochloride



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 0% at 1.0, 4% at 10.0
   and 11% at 30.0
2) TF vs. M - Inactive at 1.0,
   10.0 and 30.0
3) PPQ - 4.5 (1.0 - 19.3)
4) HP - Inactive at 20.0

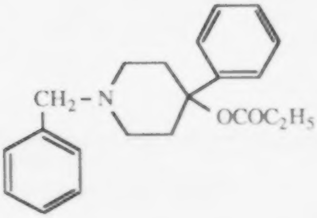
NIH 10481. Ethyl N-methyl-3α-phenyl-8-azabicyclo[3.2.1]octane carboxylate hydrochloride



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 4.0 (3.6 - 4.3)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.5 (0.2 - 1.7)

NIH 10482. 1-Benzyl-4-phenyl-4-propionoxypiperidine hydrochloride



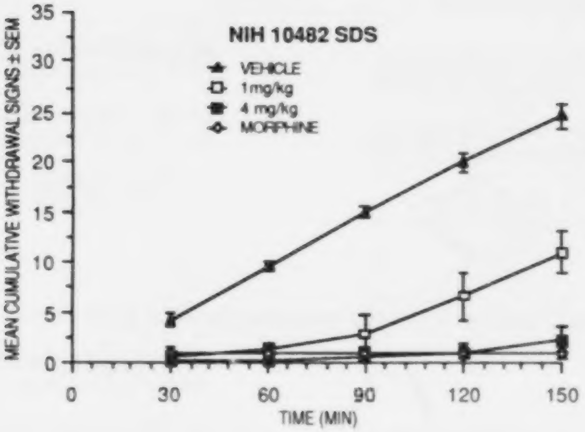
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 11.5 (6.4 - 20.6)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.4 (0.1 - 1.3)
4) HP - Inactive at 5.0, 33% at 20.0

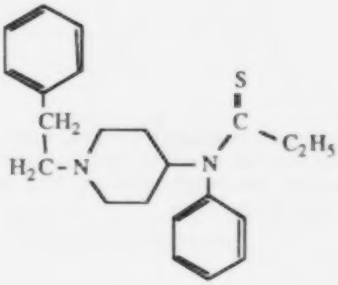
MONKEY DATA

SDS

As shown in the fig., NIH 10482 substituted completely for morphine at 4.0 mg/kg. Onset of action was rapid and duration of action equal to morphine. At the highest dose, the signs scratching, ataxia and body sag were noted. Scratching was also observed at the lower dose.



NIH 10483. N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylthiopropanamide hydrochloride



(95% C.L.) (mg/kg/s.c.)

1) TF - 0.3 (0.2 - 0.6)[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.06 (0.03 - 0.1)[a]

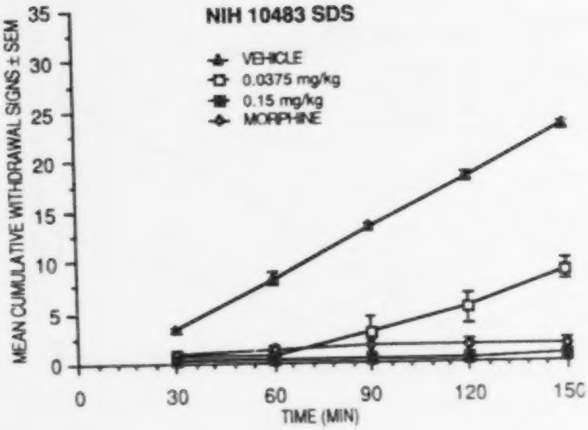
Vehicle - Tween 80 + H_20

MOUSE DATA-ED OR AD50

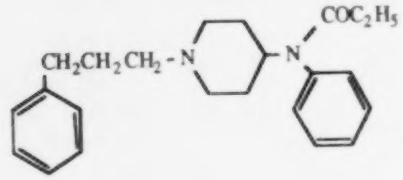
MONKEY DATA

SDS

NIH 10483 substitutes completely and promptly for morphine at 0.15 and 0.0375 mg/kg). However, at the lowest dose, the duration of action is brief (approximately 60 min). At both doses, some scratching was noted. In addition, one monkey receiving the highest dose moved about slowly. The drug is approximately 20 x more potent than morphine (see graph).



NIH 10484. N-[1-(3-Phenylpropyl)-4-piperidyl]-N-phenylpropanamide hydrochloride



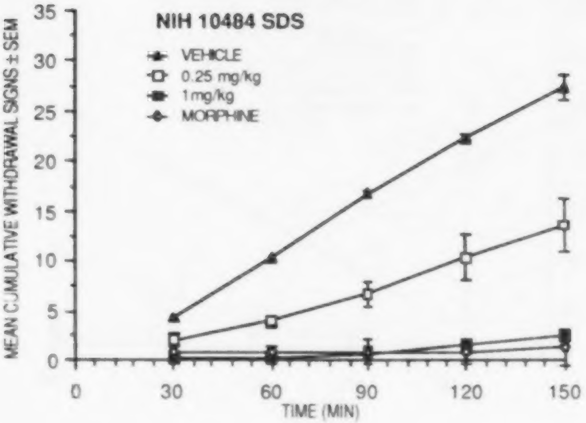
MOUSE DATA ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - 0.85 (0.6 - 1.2)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.009 (0.03 - 0.27)

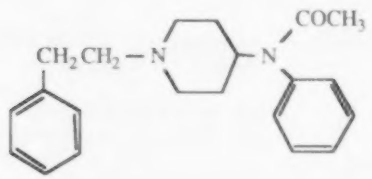
MONKEY DATA

SDS

As shown in the figure, NIH 10484 substituted completely for morphine at 1.0 mg/kg. Its potency is 3 x that of morphine and appears to have similar onset and duration of action. At the highest dose, scratching, ataxia, slowing, body and jaw sags were noted. One animals receiving the highest dose and another in the preliminary study receiving a 1.5 mg/kg cummulative dose was seen to be "staring" and "tracking".



NIH 10485, N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylacetamide hydrochloride



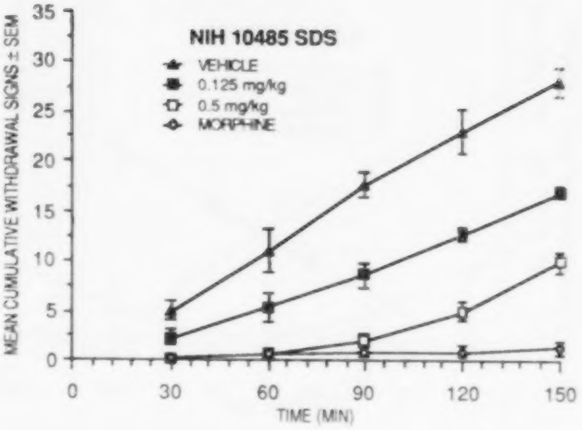
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 0.3 (0.2 - 0.5)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.05 (0.03 - 0.1)

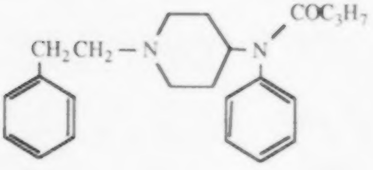
MONKEY DATA

SDS

NIH 10485 rapidly but briefly (90 min), substituted completely for morphine at 0.5 mg/kg (see figure). At peak effect, the drug is considered to be 6 times as potent as the reference standard morphine.



NIH 10486, N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylbutyramide hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

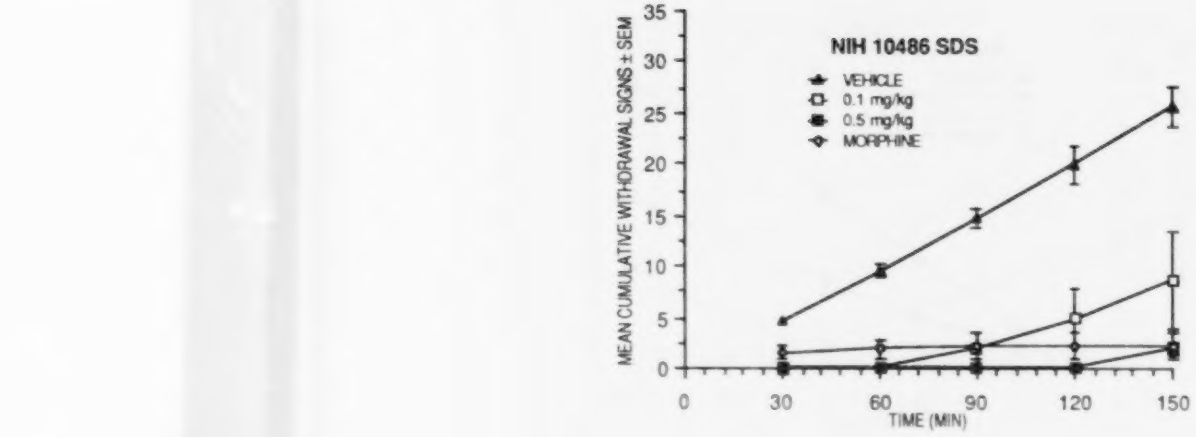
1) TF - 0.2 (0.1 - 0.5)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0.04 (0.02 - 0.08)

[a]Paralysis and Straub tail. Two mice died at 10.0 and 30.0.

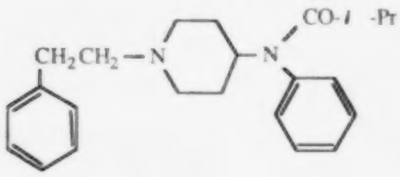
MONKEY DATA

SDS

NIH 10486 substituted completely for morphine (see fig.). The drug had a quick onset of action. Duration of action was at least 2 1/2 hr. At peak effect, the drug was 10-20X more potent than the reference standard morphine. At the highest dose, the signs jaw sag, ataxia, slowing, scratching and severe body sag were noted.



NIH 10487. N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylisobutyramide hydrochloride



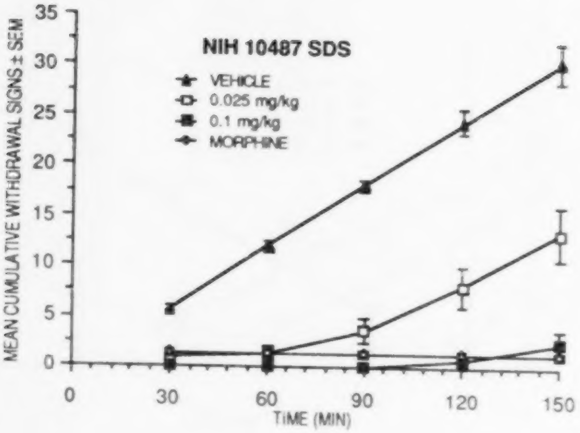
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF -0.11 (0.05 - 0.22)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.03 (0.02 - 0.03)

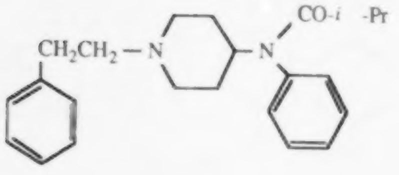
MONKEY DATA

SDS

NIH 10487 substituted completely for morphine (see graph). Potency estimate is 30 times morphine. Rapid onset and 2 1/2 hr duration of action were observed. Sagging, ataxia, slowing and scratching were noted at the highest dose during the first hr.



NIH 10488. N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylvaleramide hydrochloride.



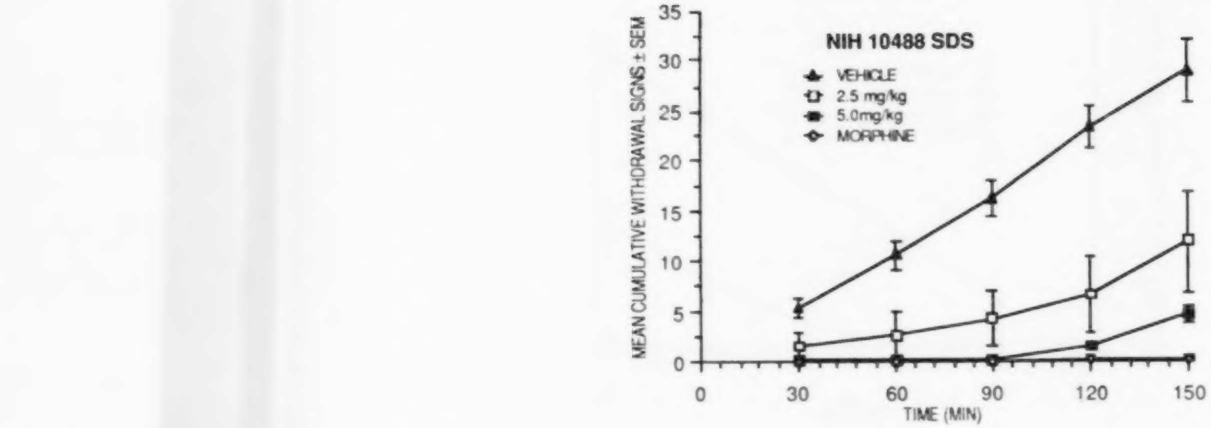
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - 5.4 (3.4 - 8.5)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 2.4 (1.2 - 4.9)

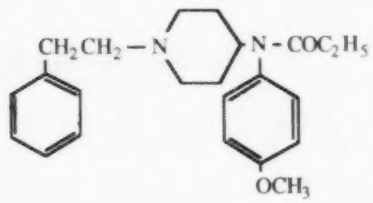
MONKEY DATA

SDS

NIH 10488 substituted completely for morphine (see graph). The drug acted promptly and at peak effect it was approximately equipotent with morphine. Its duration of action was 2 1/2 hr. One animal convulsed an hr after receiving the highest dose. The animal was given 60 mg of pentobarbital. Jaw sag and ataxia were also noted during the first 1/2 hr.



NIH 10490. N-[1-(2-Phenylethyl)-4-piperidyl]-N-(*p*-methoxyphenyl) propanamide hydrochloride



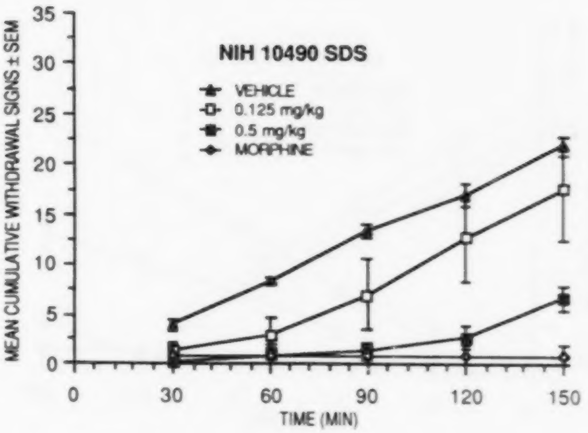
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - 0.5 (0.2 - 1.5)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.10 (0.05 - 0.17)

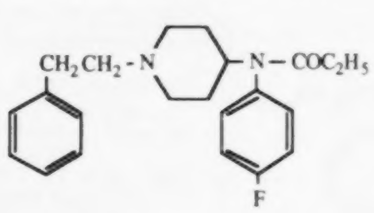
MONKEY DATA

SDS

NIH 10490 rapidly but briefly (90 min) substituted completely for morphine (see fig.). At peak effect, the drug was about 6 x or active as morphine.



NIH 10491, N-[1-(2-Phenylethyl)-4-piperidyl]-N-(*p*-fluorophenyl)propanamide hydrochloride



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

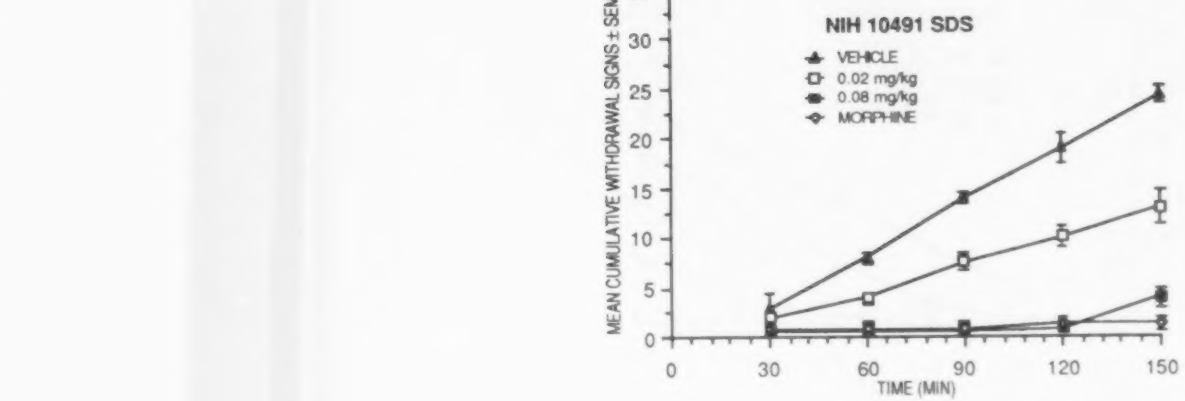
1) TF - 0.07 (0.04 - 0.11)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0.01 (0.004 - 0.025

[a] One mouse died at 30.0

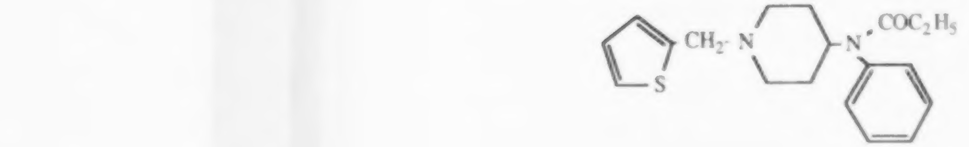
MONKEY DATA

SDS

NIH 10491 substituted completely for morphine in a dose-related manner (see fig.). The drug acted promptly but its duration of action was about 2 hr. At peak effect, this drug was 50-75 x as potent as morphine. Two animals receiving the high dose scratched frequently.



NIH 10493, N-[1-(2-Thienyl)methyl-4-piperidyl]-N-phenylpropanamide hydrochloride



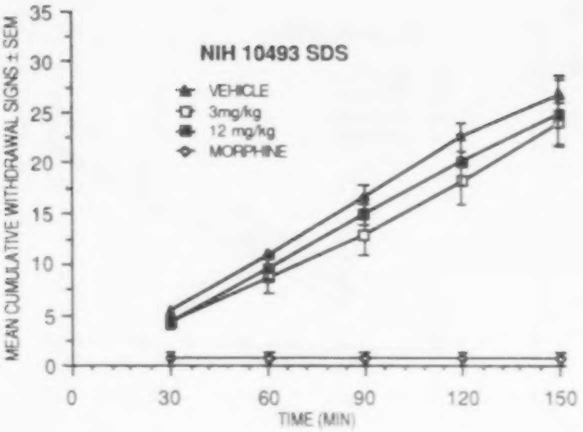
MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 9% at 1.0, 14% at 10.0 and 53% at 30.0
4) HP - Inactive at 20.0

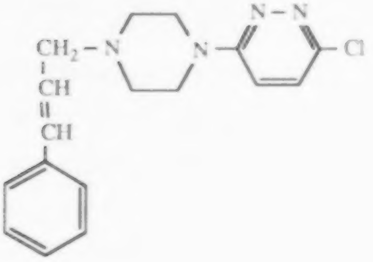
MONKEY DATA

SDS

NIH 10493 neither substituted for morphine nor exacerbated withdrawal at doses of 3.0 and 12.0 mg/kg. The drug produced effects similar to those of vehicle controls (see fig.).



NIH 10496. (E)-3-Chloro-6-[4-(3-phenyl-2-propenyl)-1-piperazinyl] pyridazine hydrochloride



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

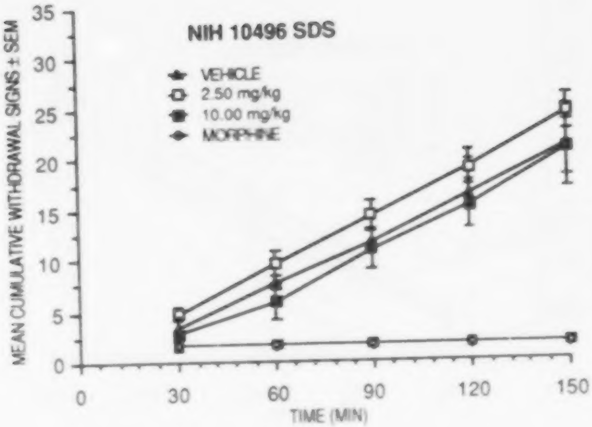
1) TF - 8% at 1.0, 17% at
10.0 and 30.5 at 30.0[a]
2) TF vs. M - Inactive at 1.0,
10.0 and 30.0
3) PPQ - 3.1 (0.8 - 12.4)
4) HP - Inactive at 20.0

[a] 30% probably due to 1 mouse that went to 10.0 sec cutoff.

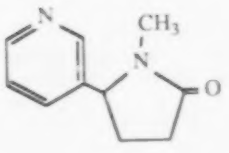
MONKEY DATA

SDS

As shown in the accompanying figure, NIH 10496 did not substitute for morphine. Note that dilute solutions of Tween 80 or DMS0 were used as vehicles.



NIH 10498. 1-Methyl-5-(3-pyridyl)-2-pyrrolidinone (Cotinine)



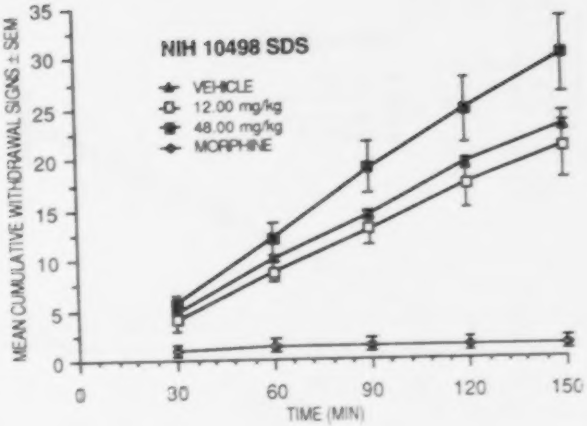
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 17.3 (5.6 - 53.6)
3) PPQ - 11% at 0.1, 40% at 1.0, 37% at 10.0 and 54% at 20.0

MONKEY DATA

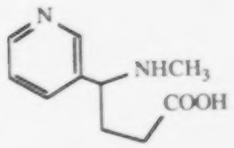
SDS

Cotinine neither attenuates nor exacerbates withdrawal in withdrawn morphine-addicted monkeys at doses of 16.0 and 48.0 mg/kg (see graph).



NIH 10499, 4-(3-Pyridyl)-4-methylaminobutyric acid

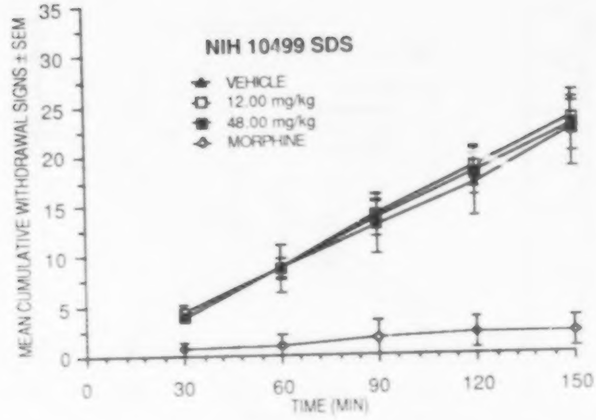
NO MOUSE DATA



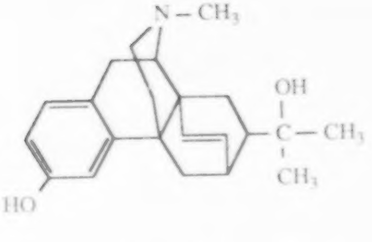
MONKEY DATA

SDS

As shown in the fig., NIH 10499 neither substituted for morphine nor exacerbated withdrawal at doses of 12.0 and 48.0 mg/kg.



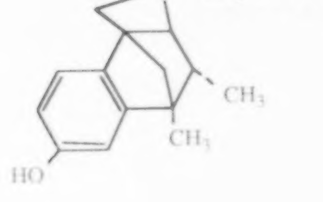
NIH 10500, 3-Hydroxy-α,α,N-trimethyl-6,14-ethenomorphinan-7β-methanol



MOUSE DATA ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 22% at 1.0, 9% at 10.0 and 45% at 30.0
3) PPQ - 0.2 (0.1 - 0.6)
4) HP - Inactive at 5.0

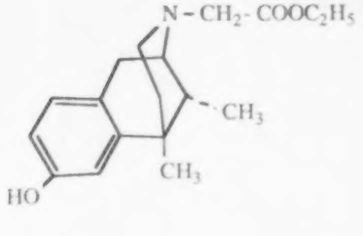
NIH 10502, (±)-2-Carboxymethyl-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 22% at 1.0, 36% at 5.0, 28% at 10.0 and 28% at 30.0
4) HP - Inactive at 20.0

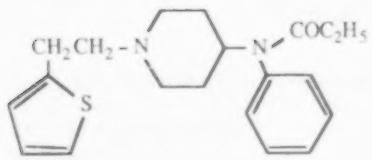
NIH 10503, (±)-2-Carbethoxymethyl-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan oxalate



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 23% at 1.0, 11% at 10.0, and 29% at 30.0
4) HP - Inactive at 20.0 and 50.0

NIH 10505, N-[1-[2-(2-Thienyl)ethyl]-4-piperidyl]-N-phenylpropanamide hydrochloride



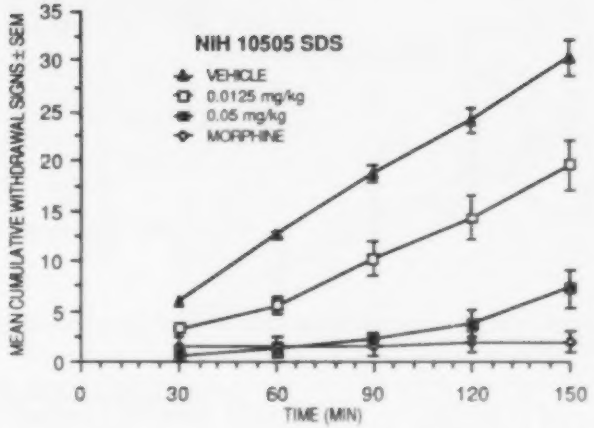
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 0.03 (0.02 - 0.05)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.02 (0.01 - 0.03)

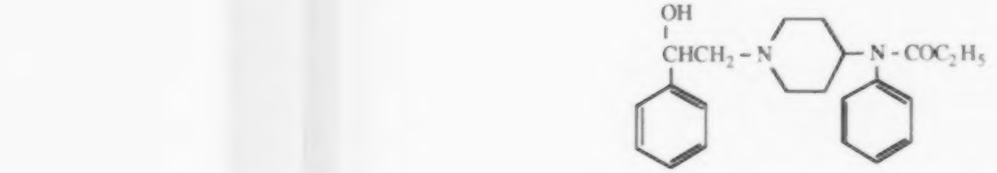
MONKEY DATA

SDS

NIH 10505 substituted completely for morphine (see fig.). Onset of action was rapid but the duration of action was 90-120 min. The drug is estimated to be 60 x as potent as morphine at peak effect.



NIH 10506. N-[1-(2-Hydroxy-2-phenylethyl)-4-piperidyl]-N-phenylpropanamide hydrochloride



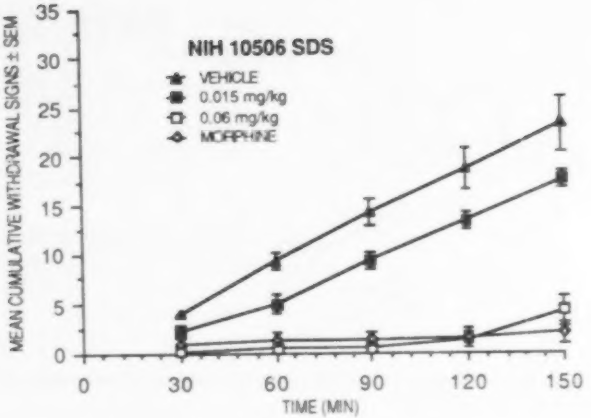
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 0.06 (0.05 - 0.09)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.01 (0.007 - 0.03)

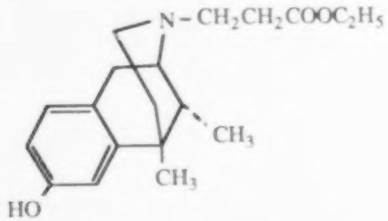
MONKEY DATA

SDS

As shown in the fig., NIH 10506 substituted completely for morphine. The drug acts promptly and its duration of action (120 - 150 min) is shorter than that of morphine. The potency estimate is 50 x morphine at peak effect dose.



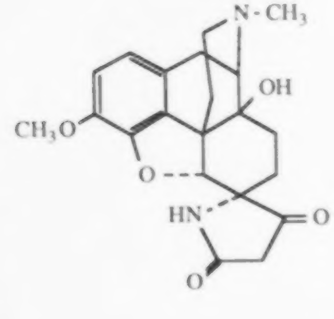
NIH 10515. (±)-2-(2-Carbethoxyethyl-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrochloride



MOUSE DATA ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 0.1, 1.0, 10.0 and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 29% at 0.01, 34% at 0.1, 46% at 1.0, 20% at 3.0 and 49% at 30.0
4) HP - Inactive at 50.0

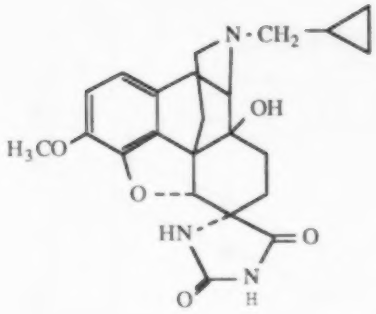
NIH 10516. Oxycodone-6-spirohydantoin



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0
2) TF vs. M - 0% at 1.0, 9% at 10.0 and 47% at 30.0
3) PPQ - 6.1 (2.2 - 17.0)

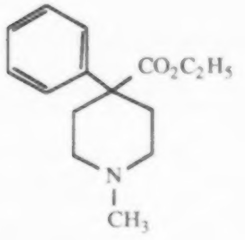
NIH 10519. Naltrexone-6-spirohydantoin succinate



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 7% at 1.0, 18% at 10.0 and 16% at 30.0
2) TF vs. M - 9.7% at 1.0, 25% at 10.0 and 33% at 30.0
3) PPQ - 22% at 1.0, 19% at 10.0 and 22% at 30.0

NIH 10522. 4-Carbethoxy-1-methyl-4-phenylpiperidine hydrochloride (pethidine hydrochloride)



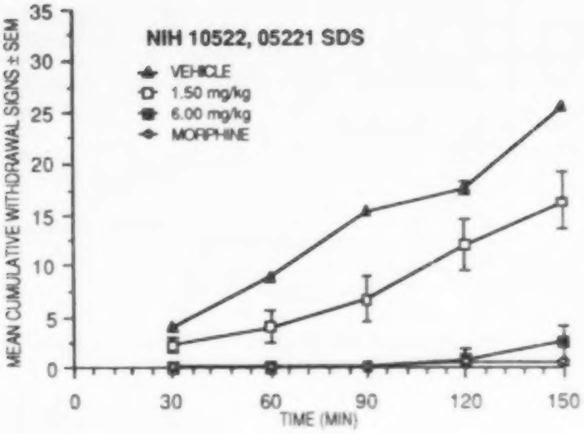
MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 7.8 (3.0 - 20.6)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 0.8 (0.3 - 2.2)

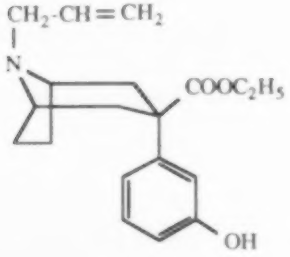
MONKEY DATA

SDS

NIH 10522 substituted completely for morphine in dose-related manner (see graph). Onset of action was prompt but duration of action was about two hours. Jaw and body sag, scratching and some "staring" were noted. At peak effect, the drug is slightly less potent than morphine.



NIH 10529. Ethyl N-allyl-3α-(3-hydroxyphenyl)-8-azabicyclo[3.2.1]octane carboxylate hydrochloride

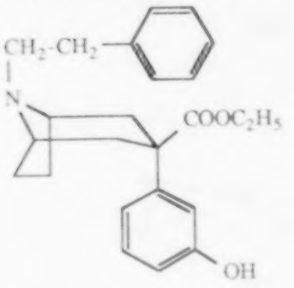


MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - Inactive at 1.0, 10.0[a] and 30.0
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 7 .7 (2.3 - 26.2)

[a] Drug supply exhausted

NIH 10530. Ethyl 3α-(3-hydroxyphenyl)-N-phenethyl-8-azabicyclo-[3.2.1.]octane carboxylate

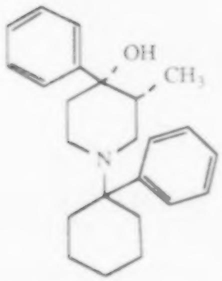


MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 2.5 (1.2 - 5.2)[a]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 1.0 (0.5 - 2.0)

[a]Vehicle - Tween 80 - H_20.

NIH 10531. 4-Hydroxy-3-methyl-4-phenyl-1-(1-phenylcyclohexyl)piperidine hydrochloride (trans 3-methyl, reference 4-phenyl)

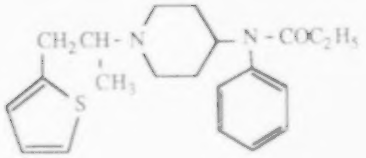


MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

1) TF - 15.3 (5.7 - 41.1)[a,b]
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 1.2 (0.5 - 3.0)

[a]Vehicle - Tween 80 + H_20.
[b]1 of 6 mice died.

NIH 10538. N-[1-[1-Methyl-2-(2-thienyl)ethyl]-4-piperidyl]-N-phenyl propanamide hydrochloride



MOUSE DATA-ED OR AD50
(95% C.L.) (mg/kg/s.c.)

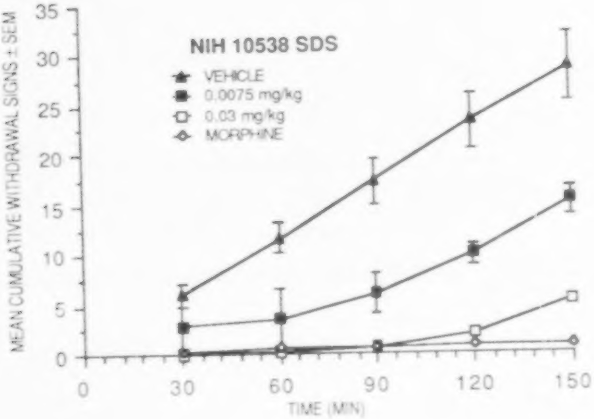
1) TF - 0.02 (0.01 - 0.05)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0[a]
3) PPQ - 0.005 (0.002 - 0.011)

[a]3 of 6 mice died at the highest dose and 1 of 6 died at the low dose.

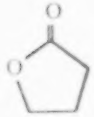
MONKEY DATA

SDS

Dose-related reduction of withdrawal signs and complete substitution for morphine were observed with NIH 10538 (see figure). Onset of action was rapid and duration was brief (about 2 hr). The drug is about 100 x more potent than morphine.



NIH 10540, γ-Butyrolactone



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

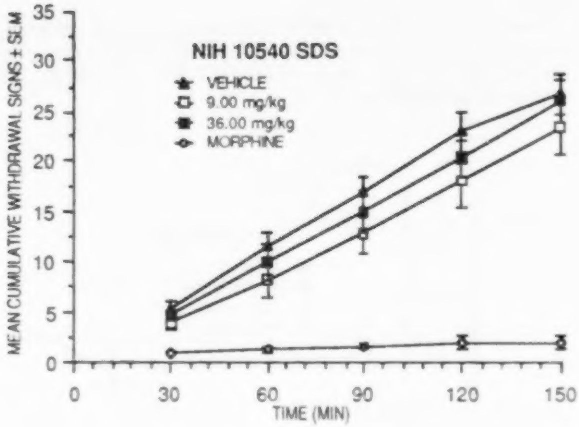
1) TF - Inactive at 96
2) TF vs. M -
3) PPQ - 7.9 (3.2 - 19.4)[a]

[a] ED80 not antagonized by 0.1, 1.0 and 10.0 mg/kg naloxone.

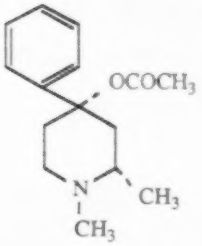
MONKEY DATA

SDS

As shown in the figure, NIH 10450 neither substituted for morphine nor exacerbated withdrawal.



NIH 10542, (+)-α-4-Acetoxy-1,2α-dimethyl-4-phenylpiperidine hydrochloride



MOUSE DATA-ED OR AD50 (95% C.L.) (mg/kg/s.c.)

1) TF - 7.5 (4.2 - 13.7)
2) TF vs. M - Inactive at 1.0, 10.0 and 30.0
3) PPQ - 4.4 (2.1 - 9.4)
4) HP - 33% at 50.0

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Evaluation of New Compounds for Opioid Activity: 1987 Annual Report

J. Woods, F. Medzihradsky, C. Smith, G. Winger
and D. Gmerek

The evaluation of new compounds by the programs at the University of Michigan and the Medical College of Virginia is coordinated by Dr. Arthur E. Jacobson, Medicinal Chemistry Section, NIAMDD, National Institutes of Health, Bethesda, MD. The drugs, which come originally from pharmaceutical companies, universites, government laboratories, and international organizations are submitted to Dr. Jacobson, who performs the MOUSE ANALGESIA tests. Values obtained in these tests for some representative opioid drugs are given in Table I.

At the UM and MCV laboratories, drug samples arrive from Dr. Jacobson with only the following information: (1) an identifying NIH number, (2) molecular weight, (3) solubility information and (4) a recommended starting dose. After the evaluation is complete and the report submitted to Dr. Jacobson, the mouse-analgesia data are released to the evaluating laboratory, and the submitter is requested to release the chemical structure within three years.

DRUG DISCRIMINATION IN RHESUS MONKEYS

We currently use two groups of monkeys to test the discriminative effects of submitted drugs. One of these groups is trained to discriminate the administration of the kappa agonist ethylketazocine (EKC). The other group is trained to discriminate the mu agonist, codeine.

The procedures used with the EKC-trained monkeys have been described by Bertalmio et al., (1982). The monkeys are removed from their home cages each day and seated in primate restraining chairs. These chairs are placed in isolation chambers equipped with two response levers, several stimulus lights and a cup to receive Noyes, banana-flavored pellets. These monkeys are required to make 100 consecutive responses on the correct one of the two levers and receive ten 300-mg food pellets. The right lever is correct if they were given a subcutaneous injection of 0.0032 mg/kg EKC immediately prior to the start of the trial. The left lever is designated correct if they were given a sham injection before the start of the trial. Each trial lasts 15 min and consists of an initial 10-min, black out period followed by a period of as long as 5 min, during which a blue light is illuminated in the chamber and the monkey can respond for food. If the food pellets are earned before the 5 min period is completed, the lights are extinguished for the remainder of this time. Typically, a daily session consists of several 15-min trials. During a training session, if EKC is given, it is given on the penultimate trial of that session. Responding on the drug-appropriate lever is reinforced during that trial and on the subsequent, final trial of the day. These last two trials may be preceded by from zero to four sham trials on a training day. A training session of six sham trials is also scheduled from time to time.

With this type of multiple, discrete-trial training, the animals can be tested with a cumulative dosing procedure. On a test session, the first trial is preceded by an injection of saline, and prior to subsequent trials, increasing, cumulative doses of the test drug are administered. One hundred consecutive responses on either lever are reinforced throughout the test session. The test drug is administered in increasing doses until the monkey either responds on the drug-appropriate lever, the response rate falls to less than half of the saline-control rate, or six trials are given. In the last situation, it is assumed that the selected dose range is too low, and the test is continued at higher doses on the next test session. Each test session is preceded and followed by a training session. The criterion for satisfactory performance must be met on each training session that is followed by a test session. This criterion is that at least 90% of the responses during each trial of a training session must be on the injection-appropriate lever, either sham or EKC.

The procedure for the codeine-trained monkeys is similar, but not identical. These animals are also trained and tested in a discrete, multiple-trial paradigm. The main difference between the codeine procedure and the EKC procedure is that the codeine monkeys are required to make 20 rather than 100 responses, and they receive a single pellet for correct responses. They can earn as many as 10 pellets during the five-minute, food-availability period of each trial, but each pellet is earned by making 20 responses. Because in this procedure, monkeys can switch from one lever to another following the delivery of food, an additional criterion is added for satisfactory performance. In addition to making 90% or more of their responses on the correct lever, the monkeys must make fewer than 40 total responses prior to earning the first food pellet of each trial. Tests of the discriminative effects of submitted drugs in the codeine-trained monkeys are also done using a cumulative-dosing procedure with dosing criteria identical to those used in the EKC-trained monkeys.

DEPENDENCE EVALUATION IN RHESUS MONKEYS

The single-dose suppression (SDS) test determines the ability of a drug to suppress the signs of withdrawal in monkeys which have been made dependent by the chronic administration of morphine (3 mg/kg every six hours). Compounds suspected of having morphine-antagonist properties are tested for their ability to precipitate the withdrawal syndrome in nonwithdrawn (NW), morphine-dependent monkeys. Nondependent monkeys (Normals) are used to determine whether the acute effects of the test drug are reversible by nalorphine or naloxone. In a primary dependence (PDS) study, non-dependent monkeys receive the test drug every six hours for 30 days to determine whether withdrawal signs will appear when the animals are challenged with an antagonist or when drug administration is discontinued.

Details of these techniques have been presented in the ANNUAL REPORT to the Committee in 1963 (Minutes of the 25th Meeting) by Deneau and Seevers (1963) and by Villarreal (1973).

SELF-ADMINISTRATION BY MONKEYS

Tests of self-administration determine the ability of the drug to maintain responding in monkeys trained to self-inject codeine. Each of at least three monkeys is studied with saline as a negative control and a number of doses of the test compound until a maximum rate of responding is obtained or until, in the absence of evidence of a reinforcing effect, observable changes in behavior are produced by the compound.

The schedule of intravenous drug delivery is a fixed-ratio 30; when a light above a lever is illuminated, the 30th response produces a five-sec, intravenous drug injection accompanied by another light that is illuminated during drug delivery. After each injection, a ten-min timeout condition is in effect, during which responses have no scheduled consequence and neither light is illuminated. Each of the two daily sessions consist of 13 injections or 130 min, whichever occurs first. Other details of the procedure and initial findings with a variety of narcotics are given in previous reports (Woods, 1977; 1980).

Doses of the drugs are typically described in terms of mg/kg/injection (inj). Duplicate observations of codeine (0.32 mg/kg/inj) and of saline are obtained for each monkey. A saline substitution is conducted before and after the series of observations on a test drug; the control rates of codeine-reinforced responding are obtained by a random sampling of two sessions interpolated between the drug-substitution sessions. These data are represented in the following graphs with individual symbols for each of the monkeys; each symbol is the mean of duplicate observations for a given dose in each monkey. The closed circles indicate the averaged data for observations on the subset of monkeys used to study each drug under each of the experimental conditions. In all cases, the rates of responding given are those calculated during only the fixed-ratio portion of each session.

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

Details of the binding assay have been described previously (Woods *et al.*, 1979; Medzihradsky *et al.*, 1984). Briefly, aliquots of a membrane preparation from rat cerebrum are incubated with ^{3}H-etorphine in the presence of 150 mM NaCl, and in the presence of different concentrations of the drug under investigation. Specific, i.e., opioid-receptor-related interaction of ^{3}H-etorphine is determined as the difference in binding obtained in the absence and presence of an appropriate excess of unlabeled etorphine. The potency of the drugs in displacing the specific binding of ^{3}H-etorphine is determined from log-probit plots of the data. It should be noted that since April 1982 the concentration of ^{3}H-etorphine in the binding assay was reduced from 3.0 nM to 0.5 nM, a concentration approaching the K_D of the radiolabeled opioid. This change was implemented in order to let the determined EC50 approximate the true K_i of a given drug. However, due to the different concentrations of the radiolabeled ligand, the EC50 determined since April, 1982 are lower than those obtained previously. For the purpose of reference, Table II contains EC50 values of representative opioids determined in binding assays using 0.5 nM ^{3}H-etorphine.

As part of our goal to develop advanced procedures to assess the interaction of newly synthesized compounds with opioid receptors (Medzihradsky, 1987), this laboratory is now in the position to determine the selectivity of ligands in binding to the mu, delta, and kappa opioid receptor. We can provide EC50 values of tested compounds in displacing the following radiolabeled opioid ligands:

etorphine (nonselective)
sufentanil (mu selective)
[D-Pen^2-D-Pen^5]enkephalin (delta selective)
U-69,593 (kappa selective)

Using these binding assays, we have described the selectivity of various established opioids in membranes from rat and monkey brain (manuscript submitted for publication).

INHIBITION OF TWITCH IN ELECTRICALLY-DRIVEN MOUSE VAS DEFERENS PREPARATIONS.

The development of new, highly selective antagonists such as the irreversible mu receptor antagonist beta-funaltrexamine (beta-FNA) and the reversible delta receptor antagonist ICI-174864 have made possible the evaluation of selectivity of opioid agonists and antagonists by use of the mouse vas deferens preparation.

Male, albino ICR mice, weighing between 25 and 30 g, are used. The mice are decapitated, the vasa deferentia removed, and 1.5 cm segments are suspended in organ baths which contain 30 ml of a modified Kreb's physiological buffer. The buffer contains the following (mM): NaCl, 118; KCl, 4.75; $CaCl_2$, 2.54; $MgSO_4$, 1.19; KH_2PO_4, 1.19; glucose, 11; $NaHCO_3$, 25; pargyline HCl, 0.3, tyrosine, 0.2; ascorbic acid, 0.1; and disodium edetate, 0.03. The buffer is saturated with 95% O_2 - 5% CO_2 and kept at 37° C. The segments are attached to strain gauge transcucers and suspended between two platinum electrodes. After a 30-min equilibration period, the segments are stimulated once every 10 sec with pairs of pulses of 2 msec duration, 1 msec apart and at supramaximal voltage.

The following antagonists are studied: naltrexone HCl, ICI-174864 [N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH] and beta-FNA. Naltrexone and ICI-174864 are added to the organ baths 15 minutes before the determination of cumulative concentration-effect relationships for the various agonists. Beta-FNA is added to the organ baths after the initial equilibration period. Thirty min later, the beta-FNA is removed from the organ baths by repeated washings with fresh buffer. The tissues are washed three times every 5 min for 15 min. Cumulative concentration-effect relationships for the various agonists are then determined 20 min after the last wash (i.e., 30 min after the beta-FNA was removed from the organ baths). EC50's are calculated by probit analysis, and pA_2 values are determined to assess relative potencies of antagonists. All drugs which are submitted for evaluation are studied in the following manner: 1) the submitted drug is tested on the vas deferens preparation in the absence and in the presence of naltrexone. The concentration of the unknown drug is varied from the lowest with activity to that which is maximally effective. 2) If the submitted drug inhibits the twitch, the ability of naltrexone to reverse the inhibition is determined. 3) The submitted drug is assessed for its ability to antagonize the actions of morphine on the vas deferens. 4) The drug is assessed for its ability to reverse the inhibition produced by a maximally effective concentration of morphine. 5) Finally, if the drug has opioid agonistic activity, studies are conducted to determine the receptor type upon which it acts. If it has antagonistic activity upon the vas deferens or upon any of the other preparations used in the Drug Evaluation Unit, the type of antagonism (competitive, noncompetitive) and the receptor selectivity is determined. For further details of the procedure see Smith (1986).

TABLE I

MOUSE ANALGESIA. Before submission to The University of Michigan, all compounds are evaluated for analgesic activity by Dr. Arthur E. Jacobson. Shown below are comparative data (ED_{50}, $\frac{mg/kg}{umol/kg}$) (95% Confidence Interval) from Hot Plate[a-c] and Nilsen[d] assays.

Compound NIH #	HOT PLATE (sc/mg/kg) (sc, umol/kg)	 (oral,mg/kg) (oral, umol/kg)	NILSEN (sc, mg/kg) (sc. umol/kg).,	 (oral, mg/kg) (oral, umol/kg)
Morphine sulfate	0.98 (0.83-1.1)	6.3 (4.7-8.3)	1.3 (1.0-1.7)	8.3 (6.0-11.4)
NIH 0001, 9929	2.9 (2.5-3.3)	18.9 (14.1-24.9)	3.9 (3.0-5.1)	24.9 (18.0-34.1)
Codeine phosphate	6.8 (4.5-10.2)	13.5 (9.7-18.7)	7.4 (4.9-11.0)	14.7 (9.2-23.3)
NIH 0002	17.1 (11.3-25.7)	34.0 (24.4-47.1)	18.6 (12.3-27.7)	37.0 (23.2-58.7)
Levorphanol tartrate	0.2 (0.1-0.3)	-	0.2 (0.16-0.3)	2.5 (1.7-3.7)
NIH 4590	0.5 (0.2-0.7)	-	0.5 (0.4-0.7)	6.2 (4.2-9.1)
Meperidine.HCl	5.3 (4.0-7.1)	-	-	-
NIH 5221	18.7 (14.1-25.0)	-	-	-
(-)-Metazocine.HBr	0.6 (0.5-0.9)	10.6 (8.0-14.1)	0.5 (0.3-0.7)	26.0 (21.0-33.0)
NIH 7569	1.9 (1.4-2.8)	34.1 (25.7-45.3)	1.6 (1.0-2.3)	83.6 (67.5-106.1)

TABLE I Continued

Dihydromorphinone.HCl NIH 0123	0.19 (0.15-0.25) 0.6 (0.5-0.8)	0.9 (0.7-1.2) 2.8 (2.2-3.7)	0.2 (0.15-0.3) 0.6 (0.5-0.9)	1.8 (1.5-2.1) 5.6 (4.7-6.5)
Nalorphine.HCl NIH 2105	9.9 (5.7-17.1) 28.4 (16.4-49.1)	- - -	23.0 (16.2-32.7) 66.1 (46.6-94.0)	- -
Cyclazocine NIH 7981	1.5 (1.1-2.1) 5.5 (4.1-7.7)	- - -	0.1 (0.07-0.16) 0.4 (0.3-0.6)	- - -
Pentazocine NIH 7958	9.3 (6.7-12.8) 32.6 (23.5-44.9)	- - -	6.5 (4.4-8.8) 22.8 (15.4-30.9)	- -
Naltrexone.HCl NIH 8503			No dose response	
Naloxone.HCl NIH 7890			No dose response	

No antinociceptive activity in hot plate assay: Phenobarbital, amobarbital, diazepam, meprobamate, mescaline, oxazepam, flurazepam.

Chloropromazine.HCl	1.1 (0.9-1.5) 3.2 (2.4-4.2)

a) Eddy and Leimbach (1953); b) Jacobson and May (1965); c) Atwell and Jacobson (1978); d) Perrine, Atwell, Tice, Jacobson and May (1972).

TABLE II

EC50 of representative opioids in displacing the specific binding of 0.5 nM ^{3}H-etorphine in a membrane preparation from rat cerebrum

Compound	EC50 (nM)		
	-NaCl	+NaCl	+Na/-Na
UM 911*	14.6	28.3	1.94
Morphine	14.0	23.6	1.69
Dextrorphan	6180	9820	1.59
UM 1071R**	1.14	1.55	1.36
Ketazocine	10.7	14.1	1.32
Ethylketazocine	5.22	6.60	1.26
(-)SKF 10047	4.09	3.93	0.96
Etorphine	0.47	0.37	0.79
(-)Cyclazocine	0.85	0.53	0.63
Naltrexone	1.43	0.63	0.44

NOTE: Binding data for these and other compounds, determined in binding assays using 3.0 nM ^{3}H-etorphine, are included in the 1978 and 1981 ANNUAL REPORTS.

*2-(3-methylfurfuryl)-2'-hydroxy-5,9α-dimethyl-6,7-benzomorphan methane sulfanate.

** IR-5R-9R-2"R-5,9-dimethyl-2'-hydroxy-2-tetrahydrofurfuryl-6,7- benzomorphan hydrochloride

SUMMARY OF TESTS PERFORMED

The compounds which were evaluated at the University of Michigan during the past year, and the individual tests which were performed are shown in Table III. Also shown are dates of Annual Reports in which results are reported of earlier tests on those compounds conducted at Michigan.

TABLE III

SUMMARY OF TESTS PERFORMED

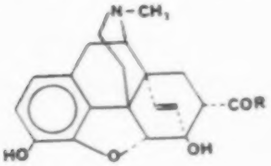
NIH	MCV	CHEMICAL CLASS AND/OR GENERIC NAME	SDS	NW	N	SA	MVD	BIND	PDS	DD	REPORT*
9834		morphine-peptide hybrid	-	-	-	+	+	+	-	-	05/16/86
10012		arylcyclohexylamine	-	-	-	-	-	-	-	+	05/16/86
10013		arylcyclohexylamine	-	-	-	-	-	-	-	+	05/16/86
10014		arylcyclohexylamine	-	-	-	-	-	-	-	+	05/16/86
10167	4359	benzomorphan	-	-	-	-	+	+	-	-	06/10/86
10318	4399	imidazolidine	+	-	-	-	1985	1985	+	-	10/09/86
10322	4404	oxymorphone	+	+	-	-	-	+	-	-	09/17/86
10324	4408	oxymorphone	+	-	-	-	-	+	-	-	09/17/86
10399	4468	oxymorphone	-	-	-	-	+	+	-	-	06/01/85
10400	4469	oxymorphone	-	-	-	-	+	+	-	-	27/01/85
10412	4479	benzofuroisoquinoline	-	-	-	-	+	+	-	-	19/11/85
10420	4478	oxymorphone	-	-	-	+	+	+	-	+	19/11/85
10426	4489	oxymorphone	-	-	-	+	+	+	-	+	27/01/86
10427	4490	oxymorphone	-	-	-	+	+	+	-	+	02/02/86
10444	4507	oxymorphone	-	-	-	-	+	+	-	-	16/05/86
10445	4506	oxymorphone	-	-	-	-	+	+	-	-	02/05/86
10462	4529	morphine	-	-	-	-	+	+	-	-	04/03/86
10463	4530	morphine	-	-	-	-	+	+	-	-	04/03/86
10464	4531	morphine	-	-	-	-	+	+	-	-	05/02/86
10465	4532	morphine	-	-	-	-	+	+	-	-	05/02/86
10466	4533	morphine	-	-	-	-	+	+	-	-	05/02/86
10467	4534	phenylpiperidine	-	-	-	-	+	+	-	-	02/05/86
10468	4535	phenylpiperidine	-	-	-	-	+	+	-	-	16/05/86
10473	4436	phenylpiperidine	-	-	-	-	+	+	-	-	07/01/87
10482	4545	phenylpiperidine	-	-	-	-	+	+	-	-	28/10/86
10483	4546	phenylpiperidine	-	-	-	-	+	+	-	-	28/10/86
10484	4547	phenylpiperidine	-	-	-	-	+	+	-	-	03/02/87
10485	4848	phenylpiperidine	-	-	-	-	+	+	-	-	03/02/87

Table III (continued)

NIH	MCV	CHEMICAL CLASS AND/OR GENERIC NAME	SDS	NW	N	SA	MVD	BIND	PDS	DD	REPORT*
10486	4849	phenylpiperidine	-	-	-	-	+	=	-	-	03/02/87
10487	4550	phenylpiperidine	-	-	-	-	+	+	-	-	03/02/87
10489	4552	phenylpiperidine	-	-	-	-	+	+	-	-	04/11/86
10490	4553	phenylpiperidine	-	-	-	-	+	+	-	-	07/01/87
10491	4554	phenylpiperidine	-	-	-	-	+	+	-	-	07/01/87
10492	4535	phenylpiperidine	-	-	-	-	+	+	-	-	03/02/87
10493	4556	thienylpiperidine	-	-	-	-	+	+	-	-	03/02/87
10496	4557	piperazinylpiperazine	-	-	-	-	+	+	-	-	03/02/87
10505	4567	thienylpiperidine	-	-	-	-	+	+	-	-	03/02/87
10506	4568	phenylpiperidine	-	-	-	-	+	+	-	-	03/02/87
10516		oxymorphone	-	-	-	-	+	+	-	-	03/02/87
10519		oxymorphone	-	-	-	-	+	+	-	-	03/02/87

* Date report was submitted to CPDD Biological Coordinator.

NIH 9834 N-(6,14-endoethano-7,8-Dihydromorphine-7μ-carbonyl)-L-phenylalanyl-L-leucinol



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 5.0 (3.6 - 6.9)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 0.30 nM in the presence of 150 mM NaCl.

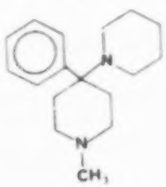
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	4.24×10^{-10}	93.7%
After naltrexone	2.61×10^{-9}	92.7%
With equimolar concentration of naltrexone	Reversal	
Equimolar concentration with morphine	No antagonism	

SUMMARY

NIH 9834 had potent opioid properties in both *in vitro* assays. (See related compounds in previous Annual Reports)

NIH 10012 1-Methyl-4-phenyl-4-(1-piperidinyl)piperidine dihydrochloride



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 40% at 100

DRUG DISCRIMINATION TEST

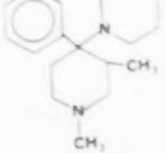
This compound was administered to three rhesus monkeys that had been trained to discriminate the stimulus effects of 1.8 mg/kg ketamine. The procedure used to train and test the monkeys has been described by Solomon *et al.*, 1982. Doses of 0.03, 0.1, 0.32 and 1.0 mg/kg were given subcutaneously to each monkey

NIH 10012 1-Methyl-4-phenyl-4-(1-piperidinyl)piperidine dihydrochloride

(continued...)

during a test session. NIH 10012 did not produce either drug-appropriate responding or response suppression in any monkey at any dose. NIH 10012, at these doses, appears to be inactive in this test of ketamine-like effects.

NIH 10013 1,3-Dimethyl-4-phenyl-4-(1-piperidinyl)piperidine dihydrochloride

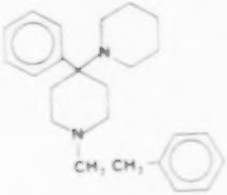


MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 56.3 (48.8-65.1)

DRUG DISCRIMINATION TEST

This compound was administered to three rhesus monkeys that had been trained to discriminate the stimulus effects of 1.8 mg/kg ketamine. The procedure used to train and test the monkeys has been described by Solomon *et al*., 1982. Doses of 0.03, 0.1, 0.32 and 1.0 mg/kg were given subcutaneously to each monkey during a test session. NIH 10013 did not produce either drug-appropriate responding or response suppression in any monkey at any dose. NIH 10013, at these doses, appears to be inactive in this test of ketamine-like effects.

NIH 10014 1-Phenethyl-4-phenyl-4-(1-piperidinyl)piperidine dihydrochloride



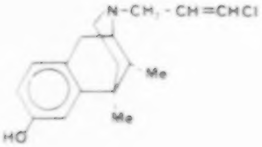
MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 50% at 50

NIH 10014 1-Phenethyl-4-phenyl-4-(1-piperidinyl)piperidine dihydrochloride

DRUG DISCRIMINATION TEST

This compound was administered to three rhesus monkeys that had been trained to discriminate the stimulus effects of 1.8 mg/kg ketamine. The procedure used to train and test the monkeys has been described by Solomon *et al.*, 1982. Doses of 0.03, 0.1, 0.32 and 1.0 mg/kg were given subcutaneously to each monkey during a test session. NIH 10014 did not produce either drug-appropriate responding or response suppression in any monkey at any dose. NIH 10014, at these doses, appears to be inactive in this test of ketamine-like effects.

NIH 10167 (+)-N-cis-3-Chloroallyl-N-normetazocine hydrobromide



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 30% at 50

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1.7 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	5.59×10^{-7}	93.0 ± 5.5%
After naltrexone	1.51×10^{-6}	100%
With equimolar concentration of naltrexone	No Reversal	
Equimolar concentration with morphine	Marked reversal	

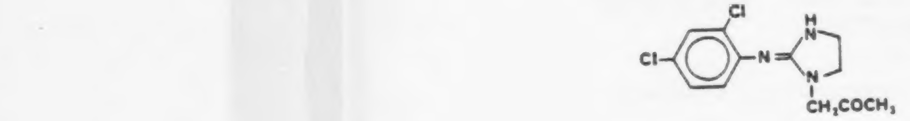
NIH 10167 (+)-N-cis-3-Chloroallyl-N-normetazocine hydrobromide

...(continued)

SUMMARY

NIH 10167 was a moderately potent opioid substance in both in vitro preparations. It appeared to have agonist-antagonist activity in the mouse, vas deferens preparation.

NIH 10318 1-Acetonyl-2-(2,4-dichlorophenyl)iminoimidazolidine. HCl



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 40% at 50

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1050 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	3.76×10^{-5}	98.5 ± 7.7%
After naltrexone	4.87×10^{-5}	97.7 ± 12.5%
With equimolar concentration of naltrexone	No Reversal	
Equimolar concentration with morphine	No reversal	

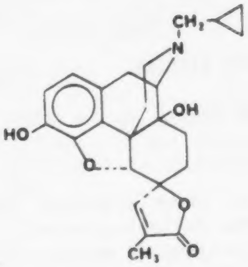
MORPHINE-DEPENDENT MONKEYS

NIH 10318 was given cumulatively to 14-hr withdrawn morphine-dependent rhesus monkeys (n=6) at the following doses: 1.0, 3.2, 10.0 and 32.0 mg/kg. There were no apparent effects of NIH 10318 on withdrawal or otherwise.

SUMMARY

NIH 10318 inhibited the twitch of the mouse vas deferens but through a non-opioid action. It displaced etorphine with a low potency. It was inactive in the SDS evaluation up to 32 mg/kg.

NIH 10322 6α-(2-Carboxy-1-propenyl)-naltrex-6β-ol γ-lactone



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 50% at 50

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 24.8 nM in presence of 150 mM NaCl.

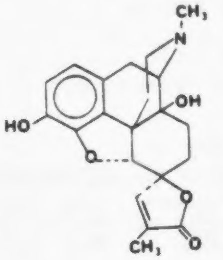
MORPHINE-DEPENDENT MONKEYS

NIH 10322 was given cumulatively to 14-hr withdrawn morphine-dependent rhesus monkeys (n=6) at the following doses: 0.1, 0.3, and 1.0 mg/kg. Withdrawal was not suppressed; it was exacerbated. Therefore, NIH 10322 was given cumulatively to non-withdrawn morphine-dependent monkeys (n=6) at the following doses: 0.1, 0.3, 1.0 and 3.2 mg/kg. NIH 10322 precipitated withdrawal in a dose-related manner. It was approximately 30 times less potent than naloxone.

SUMMARY

NIH 10322 appeared to be a less potent, naloxone-like compound.

NIH 10324 6α-(2-Carboxy-1-propenyl)-oxymorph-6β-ol γ-lactone



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: 3.5 (2.4-5.3)

best copy available

NIH 10324 6α-(2-Carboxy-1-propenyl)-oxymorph-6β-ol γ-lactone

...(continued)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 361 nM in presence of 150 mM NaCl.

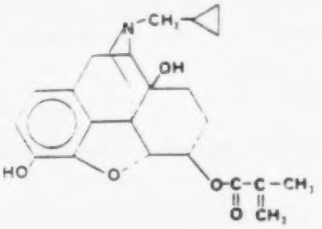
MORPHINE-DEPENDENT MONKEYS

NIH 10324 was given cumulatively to 14-hr withdrawn morphine-dependent rhesus monkeys (n=6) at the following doses: 1.0, 3.2 and 10.0 mg/kg. Two monkeys also received 32 mg/kg. Following the 3.2 mg/kg dose, the monkeys were eating and there was a slight decrease in abdominal muscle rigidity upon palpation. However, overall, there was no decrease in withdrawal. Two monkeys had muscle tremors after 10.0 mg/kg.

SUMMARY

There was an apparent discrepancy among findings with this compound. NIH 10324 needs further evaluation.

NIH 10399 6β-Naltrexol-α-methylacrylate



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive (to 20)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 0.63 nM in presence of 150 mM NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.09×10^{-5}	57.6 ± 16.8%
After naltrexone	1.22×10^{-5}	46.1 ± 3.3%
With an equimolar concentration of naltrexone	No reversal	
With an equimolar concentration of morphine	Marked reversal	

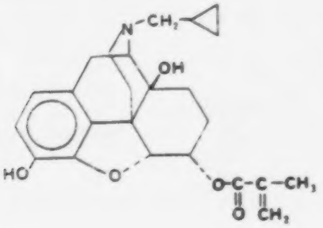
NIH 10399 6α-Naltrexol-α-methylacrylate

...(continued)

SUMMARY

NIH 10399 was an opioid antagonist upon the mouse vas deferens preparation. It was quite potent in the binding assay. It also had naltrexone-insensitive inhibitory actions in the mouse vas deferens.

NIH 10400 6 α-Naltrexol-α-methylacrylate



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 10.5 nM in presence of 150 mM NaCl.

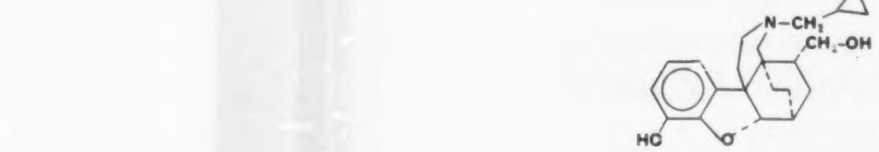
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.03×10^{-6}	100%
After naltrexone	3.13×10^{-6}	100%
With equimolar concentration of naltrexone	No reversal	
With equimolar concentration of morphine	Slight reversal	

SUMMARY

NIH 10400 appeared to be a mixed agonist-antagonist upon the isolated mouse vas deferens preparation. The estimates of potency in the two preparations were discrepant, but it should be noted that the antagonistic potency of NIH 10400 was not assessed in the mouse vas deferens. It should be noted that the compound is of quite high potency.

NIH 10412 (±)-3-Cyclopropylmethyl-1,2,3,4,5,6,7,7a-octahydro-9-hydroxy-4aH-4a,7-ethanobenzofuro(3,2-e)isoquinoline-5-methanol



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: Inactive (to 20)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 0.55 nM in presence of 150 mM NaCl

MOUSE VAS DEFERENS PREPARATION

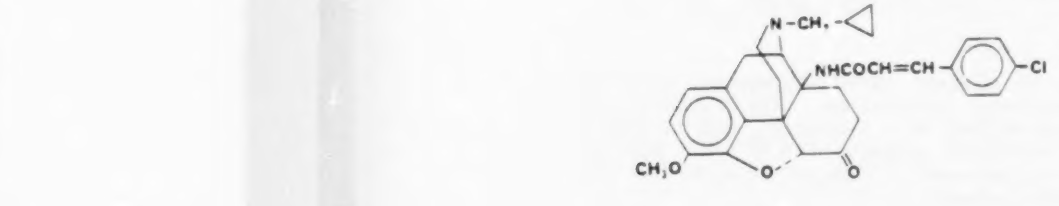
	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.5×10^{-9}	68.9 ± 5.9%
After naltrexone	3.2×10^{-8}	65.6 ± 8.0%
With equimolar concentration of naltrexone	Reversal	

SUMMARY

NIH 10412 appeared to be an opioid agonist upon the mouse vas deferens preparation equally efficacious to but more potent than morphine. The data on rat-brain binding were consistent with the findings from the smooth muscle preparation.

NIH 10420 14β-(p-Chlorocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate

MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: Inactive (to 20)



NIH 10420 14β-(p-Chlorocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate

...(continued)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1 nM in presence of 150 mM NaCl

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	5.1×10^{-9}	95.1 ± 3.2%
After naltrexone	1.4×10^{-8}	100%
After ICI-174864	2.7×10^{-8}	100%
With equimolar concentration of naltrexone	Slight reversal	
With equimolar concentration of morphine	No reversal	

SELF-ADMINISTRATION IN RHESUS MONKEYS

The reinforcing effects of NIH 10420 were evaluated in three rhesus monkeys trained to respond for 0.32 mg/kg/inj codeine (Woods, 1980). Doses of NIH 10420 were substituted for codeine in single, 130 min test sessions. Each dose was tested twice in each of the three monkeys. Rates maintained by NIH 10420 were only very slightly above those maintained by saline at the two lowest doses tested, 0.0003 and 0.001 mg/kg/inj, and at the highest dose tested, 0.01 mg/kg/inj.

Figure 1 gives details of the results of this study: 81, 1497 and 1699 are the identification numbers of the individual monkeys. Average refers to the mean of data from these three animals, while Grand Average is a historical control value for 20 monkeys under the codeine and saline conditions. The two topmost slashed horizontal lines indicate ± 3 SEM for the codeine grand average. The bottommost slashed horizontal line is + 3 SEM for the saline grand average. NIH 10420 had more prolonged effects on codeine-reinforced responding than most drugs tested in this paradigm. It required several sessions for monkeys to return to their baseline rates of codeine-reinforced responding.

NIH 10420 14β-(p-Chlorocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate

...(continued)

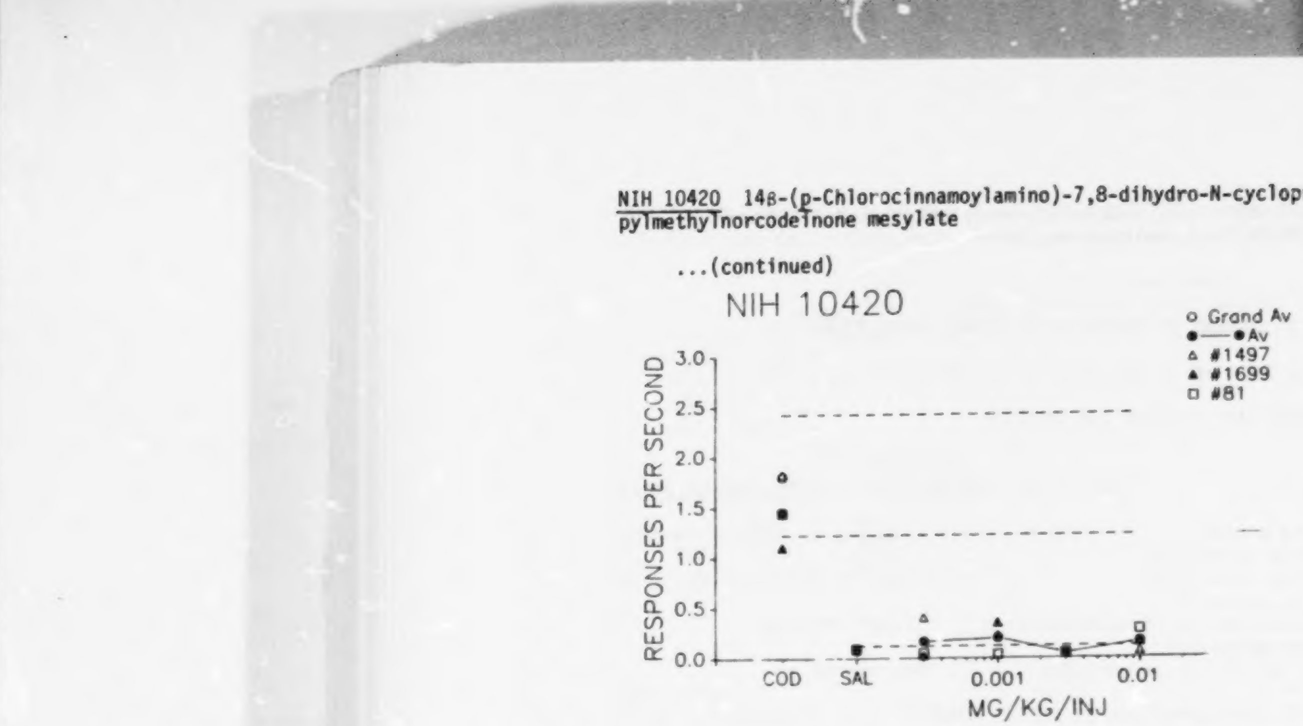


FIGURE 1

DRUG-DISCRIMINATION IN RHESUS MONKEYS

Cumulative doses of from 0.0001 to 0.1 mg/kg of NIH 10420 were evaluated in monkeys trained to discriminate 1.0 or 1.8 mg/kg codeine from sham injections in a drug-discrimination paradigm (Bertalmio *et al.*, 1982). All animals responded on the codeine-appropriate lever at the highest dose tested. One of the monkeys responded on the sham-appropriate lever on a second occasion. Thus, its average percent codeine-appropriate response was 50% (top panel, Figure 2). NIH 10420 did not produce response rate decrements at any of the doses tested (not shown).

SUMMARY

NIH 10420 has a greater relative potency to morphine in the *in vitro* preparations than in the drug discrimination assays. This is consistent with its low potency in precipitating withdrawal (MCV report). Compounds (e.g., beta-FNA and Superfit) that have "irreversible" agonist or antagonist activity have this property in common.

NIH 10420 is unusual in producing morphine-like discriminative effects without significant reinforcing effects. Compounds, e.g., l-alpha-acetyl-methadol) that have a very slow onset of behavioral action are the only compounds noted in the drug evaluation program so far that illustrate this type of dissociation.

NIH 10420 14β-(p-Chlorocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate

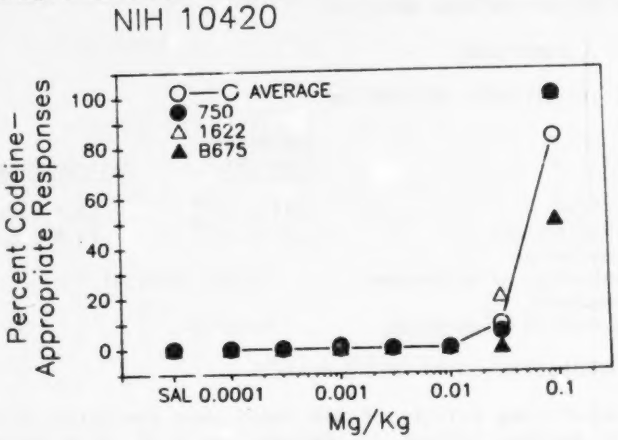
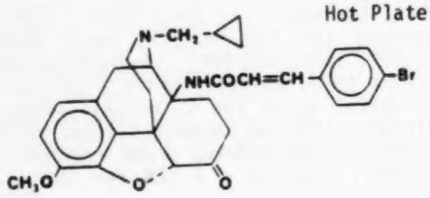


FIGURE 2

A further novel characteristic of NIH 10420 is the absence of antagonist activity in the vas deferens, but the compound precipitates abstinence in the morphine-dependent monkey. A possible explanation, of course, is the metabolic conversion of the compound to an antagonist *in vivo*. This could be assessed in the drug discrimination assay by giving NIH 10420 and assessing antagonistic activity following the cessation of agonist action.

NIH 10426 14β-(p-Bromocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate

MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive (to 20)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1.1 nM in presence of 150 mM NaCl

NIH 10426 14β-(p-Bromocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate

...(continued)

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	3.44×10^{-9}	53.6 ± 11.3%
After naltrexone	3.37×10^{-8}	73.4 ± 5.5%
With equimolar concentration of naltrexone	Slight reversal	
With equimolar concentration of morphine	Reversal	

SELF-ADMINISTRATION IN RHESUS MONKEYS .

The reinforcing effects of NIH 10426 were evaluated in three rhesus monkeys trained to respond for 0.32 mg/kg/injection codeine (Woods, J.H., 1980). Doses of NIH 10426 were substituted for codeine in single, 130 min test sessions; each test session was separated by at least three sessions with the baseline codeine dose. Each dose was tested twice in each of the three monkeys. Rates maintained by NIH 10426 were quite variable among the three monkeys. Each monkey showed rates of NIH 10426-maintained responding that were above those maintained by saline. In one monkey (1497) the rates maintained by 0.01 and 0.10 mg/kg/injection were above those maintained by codeine. In a second monkey (906) the rates maintained by 0.01 and 0.03 mg/kg/injection were as high as those maintained by codeine, and in the third monkey (1719), 0.03 and 1.0 mg/kg/injection maintained the highest rates, and these were considerably below the rates maintained by codeine. (This monkey had lower codeine-reinforced rates than did the other monkeys).

Figure 1 gives details of the results of this study. The data from the individual monkeys are indicated by each animal's identification numbers (1497, 1719, and 906). Average refers to the mean of data from these three animals, while Grand Average refers to a historical control value for 20 monkeys under the codeine and saline conditions. The two topmost slashed horizontal lines indicate + 3 SEM for the codeine grand average. The bottommost slashed horizontal line is + 3 SEM for the saline grand average.

NIH 10426 14β-(p-Bromocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate

...(continued)

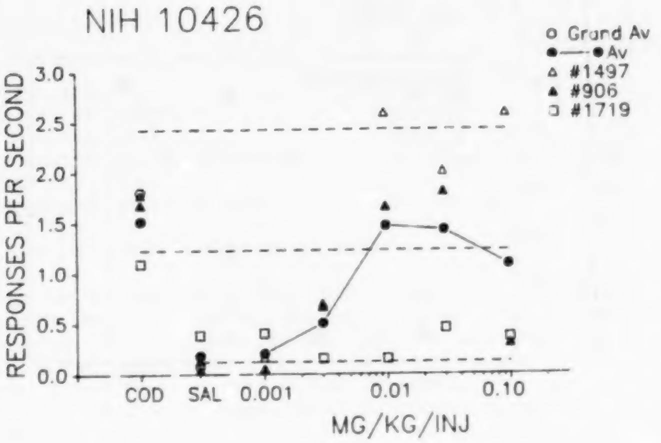


FIGURE 1

NIH 10426 had more prolonged effects on codeine-reinforced responding than most drugs tested in this paradigm. It required several sessions for two of the monkeys to return to their baseline rates of codeine-reinforced responding.

DRUG-DISCRIMINATION IN RHESUS MONKEYS

Cumulative doses of from 0.01 to 0.10 mg/kg NIH 10426 were evaluated in the monkeys trained to discriminate 1.0 or 1.8 mg/kg codeine from sham injections in a drug-discrimination paradigm (Bertalmio et al., 1982). All three monkeys responded on the codeine-appropriate lever at a cumulative dose of 0.10 mg/kg (Figure 2). NIH 10426 did not produce any response decrements at any of the doses tested (not shown).

SUMMARY

NIH 10426 was a potent opiate agonist upon both the *in vitro* assays. A novel finding was that the compound might have significant kappa antagonist activity. The antagonist activity

NIH 10426 14β-(p-Bromocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate

...(continued)

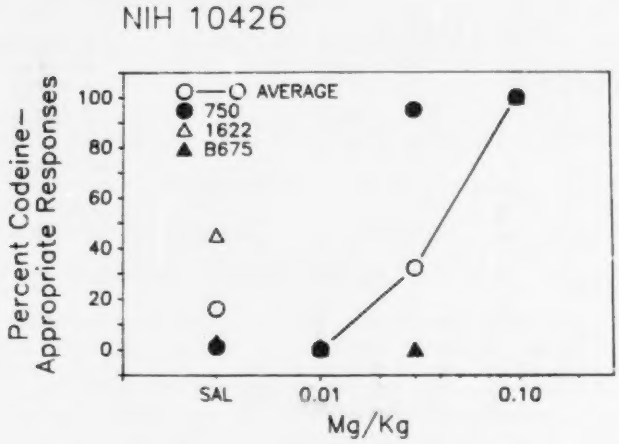
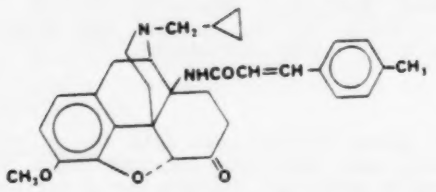


FIGURE 2

of NIH 10426 may be difficult to study in vivo since the concentrations that exert this action also exert agonist activity. It had morphine-like activity in the drug-discrimination and drug-reinforcement studies.

NIH 10427 14β-(p-Methylcinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate

MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive (to 20)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 4.5 nM in presence of 150 mM NaCl

NIH 10427 14β-(p-Methylcinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate

...(continued)

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.60×10^{-8}	62.4 ± 13.7%
After naltrexone	1.04×10^{-7}	69.0 ± 6.7%
With equimolar concentration of naltrexone	Reversal	
With equimolar concentration of morphine	No reversal	

SELF-ADMINISTRATION IN RHESUS MONKEYS

The reinforcing effects of NIH 10427 were evaluated in three rhesus monkeys trained to respond for 0.32 mg/kg/injection codeine (Woods, J.H., 1980). Doses of NIH 10427 were substituted for codeine in single, 130 min test sessions. Each dose was tested twice in each of the three monkeys. Rates maintained by NIH 10427 were below those maintained by codeine, but above those maintained by saline. The highest average rate of responding was approximately half way between these points and occurred at a dose of 0.01 mg/kg/inj.

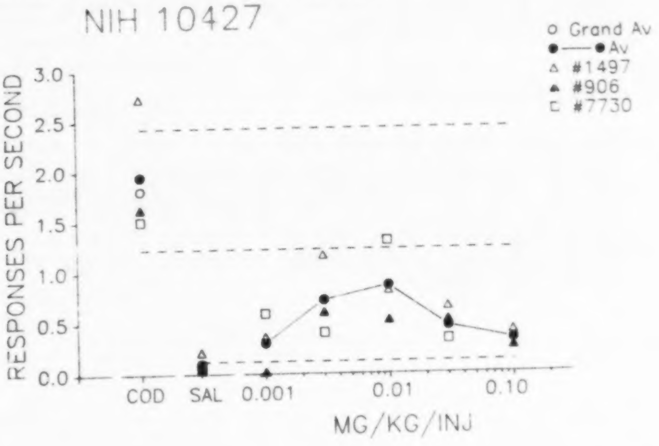


FIGURE 1

NIH 10427 14β-(p-Methylcinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate

....(continued)

Figure 1 gives details of the results of this study: 1497, 906 and 7730 are identification numbers of individual monkeys. Average refers to the mean of data from these three animals, while Grand Average refers to a historical control value for 20 monkeys under the codeine and saline conditions. The two topmost slashed horizontal lines indicate ± 3 SEM for the codeine grand average. The bottom most slashed horizontal line is + 3 SEM for the saline grand average.

There was more variability than usual in the rates of responding maintained by the three monkeys at each dose. There was also a large amount of variability within each monkey at each dose. This is not shown on the attached figure, but was much larger than we typically see with drugs that act as reinforcers in this paradigm. NIH 10427 also had more prolonged effects on codeine-reinforced responding than most other drugs evaluated. It required several sessions for monkeys to return to their baseline rates of codeine-reinforced responding. On occasion, 0.32 mg/kg cocaine was substituted for the baseline dose of 0.32 mg/kg codeine when, following administration of NIH 10427, the opiate was not maintaining high rates of responding. The cocaine invariably was able to maintain high rates of responding even when codeine was not.

DRUG-DISCRIMINATION STUDIES

Cumulative doses of from 0.03 to 3.2 mg/kg of NIH 10427 were evaluated in monkeys trained to discriminate 1.0 or 1.8 mg/kg codeine from sham injections in a drug-discrimination paradigm (Bertalmio *et al.*, 1982). All animals responded on the codeine-appropriate lever at a dose of 0.3 mg/kg (Figure 2). NIH 10427 did not produce any response decrements at any of the doses tested (not shown).

NIH 10427 14β-(p-Methylcinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnorcodeinone mesylate

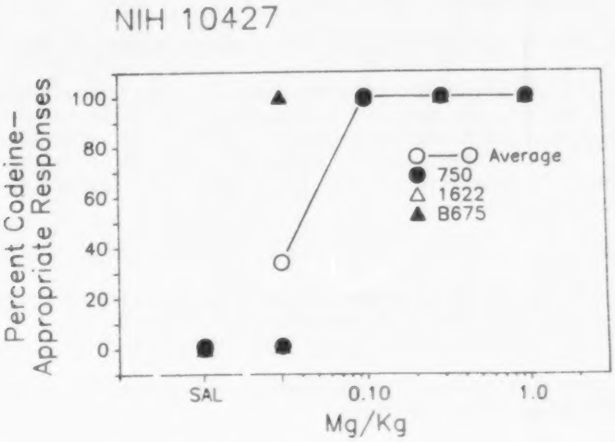


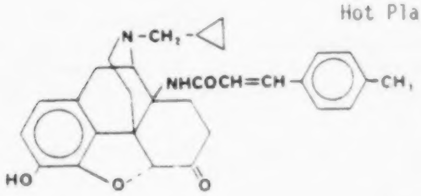
FIGURE 2

SUMMARY

NIH 10427 was a morphine-like agonist in behavioral assays; it was 10 times as potent. It was different from morphine in that it suppressed self-injection responding for an extended period after its administration. In both in vitro assays it had potent opioid activity. It was also an agonist in the mouse vas deferens preparation.

NIH 10444 14β-(p-Methylcinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnormorphinone mesylate

MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive (to 20)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 0.79 nM in presence of 150 mM NaCl

NIH 10444 14β-(p-Methylcinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnormorphinone mesylate

...(continued)

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

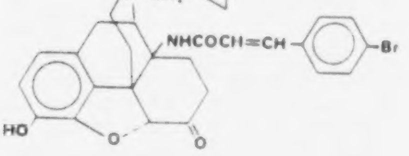
At no concentration did NIH 10444 cause a significant inhibition of the twitch. It markedly antagonized the inhibitory actions of sufentanil, a potent mu receptor agonist. At a concentration of 10^{-7} M, NIH 10444 caused a shift to the right in the sufentanil concentration-effect curve and reduced the maximum response to 15% of control value.

SUMMARY

NIH 10444 was a highly potent compound in the binding assay, and to be a pure antagonist in the mouse vas deferens.

NIH 10445 14β-(p-Bromocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnormorphinone mesylate

MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive (to 20)



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 0.79 nM in presence of 150 mM NaCl

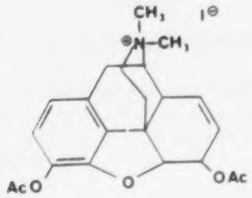
INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

At no concentration did NIH 10445 cause a significant inhibition of the twitch. It markedly antagonized the inhibitory actions of sufentanil, a potent mu receptor agonist. At a concentration of 10^{-7} M, NIH 10445 abolished all responses to sufentanil when studied in concentrations up to 10^{-4} M.

SUMMARY

NIH 10445 was a highly potent opioid in the binding assay and a quite "pure" antagonist in the mouse vas deferens.

NIH 10462 Heroin methiodide



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 > 10 μM (23.6% inhibition at 10 μM).

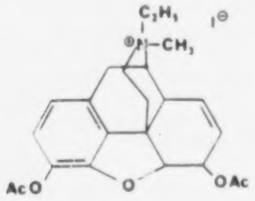
INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.31×10^{-7}	32.2% ± 6.6
After naltrexone	5.97×10^{-8}	26.5% ± 3.6
With equimolar concentration of naltrexone	Slight reversal	
With equimolar concentration of sufentanil	No reversal	

SUMMARY

NIH 10462 failed to significantly displace etorphine, but may have some weak opioid activity in the mouse vas deferens.

NIH 10463 Heroin ethiodide



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 20

NIH 10463 Heroin ethiodide

...(continued)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 10 μM in the presence of NaCl (15.2% inhibition at 10 μM).

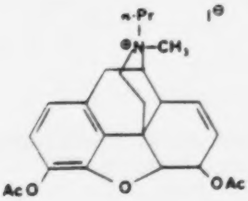
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.82×10^{-8}	48.2% ± 7.4
After naltrexone	7.48×10^{-8}	26.2% ± 3.47
With equimolar concentration of naltrexone	Slight reversal	
Equimolar concentration of sufentanil	No reversal	

SUMMARY

NIH 10463 probably has insignificant opioid agonist activity in view of the results of both assay systems. Standards of reference (e.g., morphine, U-50,488, or enkephalin analogues) suppress the twitch of the vas deferens to a greater extent than NIH 10463.

NIH 10464 Heroin propiodide



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 > 10 μM in the presence of NaCl (20% inhibition at 6 μM).

NIH 10464 Heroin propiodide

...(continued)

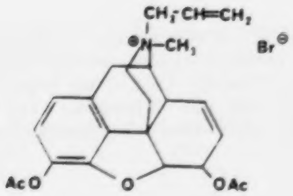
MOUSE VAS DEFERENS PREPARATION

No concentration of this drug significantly inhibited the contractions of the vas deferens. In the presence of NIH 10464, 100 nM, the sufentanil concentration-effect curve was not altered.

SUMMARY

NIH 10464 was without significant opioid activity in either assay.

NIH 10465 Heroin allobromide



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 10 μM in the presence of NaCl (20% inhibition at 6 μM).

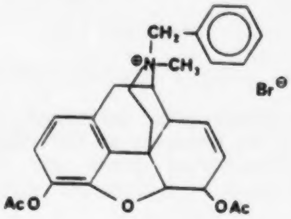
MOUSE VAS DEFERENS PREPARATION

NIH 10465 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-10} M to 10^{-4} M. No concentration of this drug significantly inhibited the contractions of the vas deferens. In the presence of NIH 10465, 100 nM, the sufentanil concentration-effect curve was not altered.

SUMMARY

NIH 10465 was without significant opioid activity in either assay.

NIH 10466 Heroin benzobromide



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 10 μM in the presence of NaCl (18% inhibition at 6 μM).

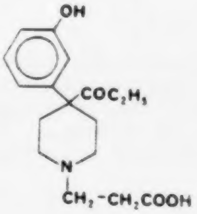
MOUSE VAS DEFERENS PREPARATION

NIH 10466 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-10} M to 10^{-4} M. No concentration of this drug significantly inhibited the contractions of the vas deferens. In the presence of NIH 10466, 100 nM, the sufentanil concentration-effect curve was not altered.

SUMMARY

NIH 10466 was without significant opioid activity in either assay.

NIH 10467 N-2-(Carboxyethyl)-N-norketobemidone hydrochloride



MOUSE ANALGESIA, ED50, (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 10 μM in the presence of NaCl (36% inhibition at 6 μM).

NIH 10467 N-2-(Carboxyethyl)-N-norketobemidone hydrochloride

...(continued)

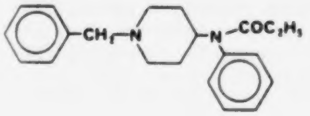
MOUSE VAS DEFERENS PREPARATION

NIH 10467 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-10} M to 10^{-4} M. No concentration of this drug significantly inhibited the contractions of the vas deferens. In the presence of NIH 10467, 100 nM, the sufentanil concentration-effect curve was not altered.

SUMMARY

NIH 10467 was without significant opioid activity in either assay.

NIH 10468 N-(1-Benzyl-4-piperidyl)-N-phenylpropanamide hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 2200 nM in presence of 150 mM NaCl.

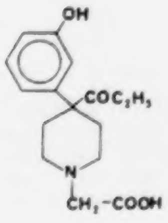
MOUSE VAS DEFERENS PREPARATION

NIH 10468 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-10} M to 10^{-4} M. No concentration of this drug significantly inhibited the concentration of the vas deferens.

SUMMARY

NIH 10468 failed to have significant opioid activity in either preparation; there was a suggestion of slight antagonist activity in the vas deferens, but the compound had very low potency in the binding assay.

NIH 10473 -Carboxymethyl-N-norketobemidone hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 10 µM in the presence of NaCl (32.8% inhibition at 6 µM).

MOUSE VAS DEFERENS PREPARATION

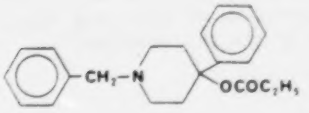
NIH 10473 was studied upon the isolated, electrically stimulated mouse vas deferens preparation in concentrations which ranged from 10^{-10} M to 10^{-4} M. No concentration of this drug significantly inhibited the contractions of the vas deferens. In the presence of NIH 10473, 100 nM, the sufentanil concentration-effect curve was not altered.

SUMMARY

NIH 10473 was devoid of activity upon opioid receptors in both preparations.

NIH 10482 1-Benzyl-4-phenyl-4-propionoxypiperidine hydrochloride

MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: 33% at 20



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 472 nM in presence of NaCl.

NIH 10482 1-Benzyl-4-phenyl-4-propionoxypiperidine hydrochloride

...(continued)

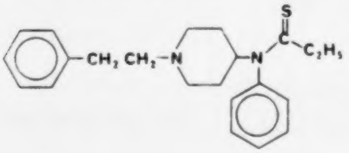
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.09×10^{-6}	86.7 ± 7.4%
After naltrexone	Complete blockade	(0)
After ICI-174864	4.85×10^{-7}	68.7 ± 29.3%
After beta-funaltrexamine	Complete blockade	(0)
With equimolar concentration of naltrexone	Reversal	
Equimolar concentration with sufentanil	Did not alter response	

SUMMARY

NIH 10482 appeared to be a less potent mu receptor agonist compared to morphine upon the mouse vas deferens preparation. This was consistent with its potency in the binding assay.

NIH 10483 N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylthiopropanamide hydrochloride



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Insoluble

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 13.1 nM in presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.76×10^{-7}	100%
After naltrexone	1.45×10^{-5}	94.2 ± 5.8%
After ICI-174864	2.52×10^{-8}	100%
After beta-funaltrexamine	9.19×10^{-8}	63.3 ± 24%
With equimolar concentration of naltrexone	Reversal	
Equimolar concentration with sufentanil	Did not alter response	

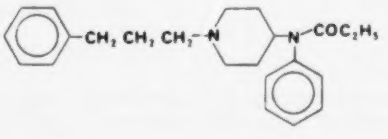
NIH 10483 N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylthiopropanamide hydrochloride

...(continued)

SUMMARY

NIH 10483 appeared to be a mu receptor agonist similar to morphine upon the mouse vas deferens preparation. It was more potent in the binding assay than morphine.

NIH 10484 N-[1-(3-Phenylpropyl)-4-piperidyl]-N-phenylpropanamide hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 78.2 nM in the presence of NaCl.

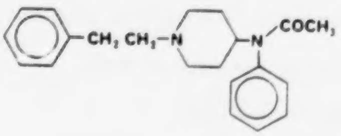
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.30×10^{-7}	49.9 ± 11%
After naltrexone	Complete blockade	(0)
After ICI-174864	9.82×10^{-8}	35.6 ± 11.2%
After beta-funaltrexamine	Complete blockade	(0)
With equimolar concentration of naltrexone	Reversal	
Equimolar concentration with sufentanil	Did not alter response	

SUMMARY

NIH 10484 appeared to be a mu receptor agonist similar to morphine upon the mouse vas deferens preparation. It is somewhat less potent in the binding assay than morphine.

NIH 10485 N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylacetamide hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 676 nM in the presence of NaCl.

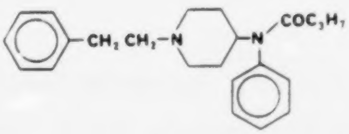
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	4.42×10^{-7}	100%
After naltrexone	4.70×10^{-6}	33.7 ± 3.3%
After ICI-174864	5.17×10^{-7}	100%
After beta-funaltrexamine	4.80×10^{-7}	29.3 ± 2.1%
With equimolar concentration of naltrexone	Reversal	
Equimolar concentration with sufentanil	Did not alter response	

SUMMARY

NIH 10485 appeared to be a mu receptor agonist similar to morphine upon the mouse vas deferens preparation; it was much less potent than morphine in both assays.

NIH 10486 N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylbutyramide hydrochloride



NIH 10486 N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylbutyramide hydrochloride

...(continued)

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 58.7 nM in the presence of NaCl.

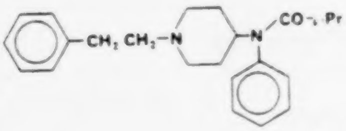
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.11×10^{-7}	71.5 ± 11.7%
After naltrexone	2.11×10^{-6}	82.7 ± 17.3%
After ICI-174864	1.88×10^{-7}	45.1 ± 21.8%
After beta-funaltrexamine	2.19×10^{-7}	46.9 ± 9.6%
With equimolar concentration of naltrexone	Reversal	
Equimolar concentration with sufentanil	Did not alter response	

SUMMARY

NIH 10486 appeared to be a mu receptor agonist similar to morphine upon the mouse vas deferens preparation. The binding and smooth muscle assessments of NIH 10486 was congruent.

NIH 10487 N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylisobutyramide hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 85.1 nM in the presence of NaCl.

NIH 10487 N-[1-(2-Phenylethyl)-4-piperidyl]-N-phenylisobutyramide hydrochloride

...(continued)

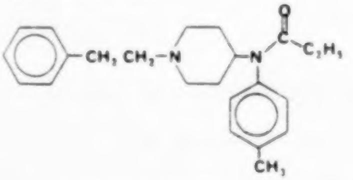
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	5.89×10^{-8}	99.35 ± 0.3%
After naltrexone	4.84×10^{-6}	100%
After ICI-174864	1.64×10^{-8}	95.0 ± 3.1%
After beta-funaltrexamine	2.98×10^{-7}	44.8 ± 12.8%
With equimolar concentration of naltrexone	Reversal	
Equimolar concentration with sufentanil	Did not alter response	

SUMMARY

NIH 10487 appeared to be a mu receptor agonist similar to morphine upon the mouse vas deferens preparation; its potency in the binding assay was similar to that of morphine as well.

NIH 10489 N-[1-(2-Phenylethyl)-4-piperidyl]-N-(p-methylphenyl) propanamide hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 85.1 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	4.30×10^{-7}	99.6 ± 4.0%
After naltrexone	8.96×10^{-6}	39.0 ± 3.1%
After ICI-174864	8.66×10^{-7}	100%
After beta-funaltrexamine	4.27×10^{-5}	96.5 ± 1.8%
With equimolar concentration of naltrexone	No reversal	
Equimolar concentration with sufentanil	Did not alter response	

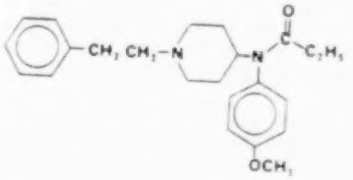
NIH 10489 N-[1-(2-Phenylethyl)-4-piperidyl]-N-(p-methylphenyl) propanamide hydrochloride

...(continued)

SUMMARY

NIH 10489 appeared to be a mu receptor agonist upon the mouse vas deferens preparation. It was close to morphine in potency in the binding assay, and somewhat less potent than morphine in the mouse vas deferens. The observation that beta-funaltrexamine did not diminish the maximum response to NIH 10489 suggested that a component of the action of NIH 10489 upon the vas deferens was mediated either by kappa receptors or by a non-opiate mechanisms.

NIH 10490 N-[1-(2-Phenylethyl)-4-piperidyl]-N-(p-methoxyphenyl) -propanamide hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 90.7 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.09×10^{-7}	98.9 ± 0.8%
After naltrexone	2.40×10^{-5}	97.4 ± 1.3%
With ICI 174,864	2.79×10^{-7}	99.4 ± 0.6%
With beta-funaltrexamine	9.99×10^{-7}	43.7 ± 7.3%
With equimolar concentration of naltrexone	Did not alter response	
Equimolar concentration of sufentanil	No reversal	

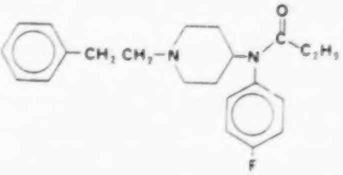
NIH 10490 N-[1-(2-Phenylethyl)-4-piperidyl]-N-(p-methoxyphenyl) -propanamide hydrochloride

...(continued)

SUMMARY

NIH 10490 appeared to be a relatively selective mu receptor agonist upon the mouse vas deferens preparation. Its potency in both preparations was somewhat less than that of morphine.

NIH 10491 N-[1-(2-Phenylethyl)-4-piperidyl]-N-(p-fluorophenyl) propanamide hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 16.4 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

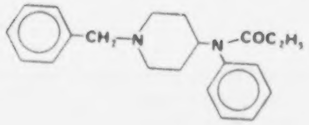
	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.20×10^{-8}	95.3 ± 1.5%
After naltrexone	1.01×10^{-6}	100%
After ICI 174864	5.95×10^{-8}	83.1 ± 7.8%
After beta-funaltrexamine	1.14×10^{-7}	81.3 ± 7.3%
With equimolar concentration of naltrexone	Did not alter response	
Equimolar concentration of sufentanil	No reversal	

SUMMARY

NIH 10491 appeared to act primarily as a mu receptor agonist upon the mouse vas deferens preparation although actions upon the kappa and delta receptors cannot be ruled out. NIH 10491 was as potent as morphine in both preparations.

NIH 10492 see NIH 10468

MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 30



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 2310 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

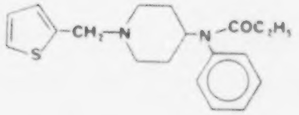
NIH 10492 had no significant opiate activity on the mouse vas deferens preparation.

SUMMARY

NIH 10492 was without significant opioid activity in both *in vivo* preparations.

NIH 10493 N-[1-(2-Thienyl)methyl-4-piperidyl]-N-phenylpropanamide hydrochloride

MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 30



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 2480 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

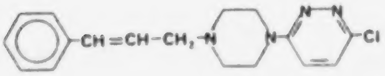
NIH 10493 had no significant activity upon the mouse vas deferens preparation.

SUMMARY

NIH 10493 was without significant opioid activity in both *in vivo* preparations.

NIH 10496 (E)-3-Chloro-6-[4-(3-phenyl-2-propenyl)-1-piperazinyl] pyridazine hydrochloride

MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 20



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1800 nM in the presence of NaCl.

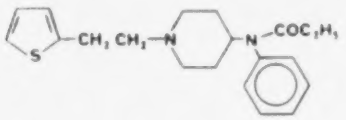
MOUSE VAS DEFERENS PREPARATION

NIH 10496 was inactive upon the isolated, electrically stimulated mouse vas deferens preparation. It neither altered responses to sufentanil, nor reversed the inhibitory effects of sufentanil.

SUMMARY

NIH 10496 did not have significant opiate activity in both *in vivo* preparations.

NIH 10505 N-[1-[2-(2-Thienyl)ethyl]-4-piperidyl]-N-phenylpropanamide hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 45.8 nM in the presence of NaCl.

NIH 10505 N-[1-[2-(2-Thienyl)ethyl]-4-piperidyl]-N-phenylpropanamide hydrochloride

...(continued)

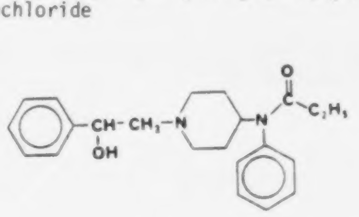
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	1.60×10^{-8}	98.4 ± 1.62%
After naltrexone	1.65×10^{-6}	93.6 ± 3.86%
After ICI 174864	1.27×10^{-8}	99.3 ± 0.7%
After beta-funaltrexamine	4.57×10^{-7}	49.5 ± 6.92%
With equimolar concentration of sufentanil	No reversal	

SUMMARY

NIH 10505 appeared to be a relatively selective mu receptor agonist upon the mouse vas deferens preparation. The binding assay suggested NIH 10505 to be less potent than morphine; while the reverse was true with the mouse vas deferens.

NIH 10506 N-[1-(2-Hydroxy-2-phenylethyl)-4-piperidyl]-N-phenylpropanamide hydrochloride



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 80.0 nM in the presence of NaCl.

NIH 10506 N-[1-(2-Hydroxy-2-phenylethyl)-4-piperidyl]-N-phenylpropanamide hydrochloride

...(continued)

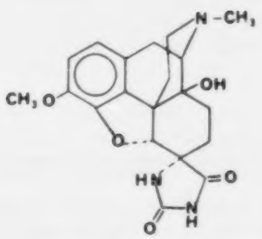
MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.71 x 10^{-8}	100%
After naltrexone	3.31 x 10^{-6}	97.4 ± 1.29%
After ICI 174864	2.31 x 10^{-8}	100%
After beta-funaltrexamine	1.34 x 10^{-6}	87.6 ± 7.53%
With equimolar concentration of sufentanil	No reversal	

SUMMARY

NIH 10506' appeared to be a relatively selective mu receptor agonist upon the mouse vas deferens preparation. In binding to opiate receptor in brain membranes, its EC50 was approximately 3-fold higher than that of morphine.

NIH 10516 Oxycodone-6-spirohydantoin



MOUSE ANALGESIA, ED50 (mg/kg)
Hot plate: Inactive at 20

DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of > 6000 nM (3.1% inhibition at 5 μM) in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

NIH 10516 was inactive upon the mouse vas deferens preparation in concentrations which ranged from 10^{-9} M to 3 x 10^{-5} M. It neither altered responses to sufentanil nor reversed the inhibitory effects of sufentanil.

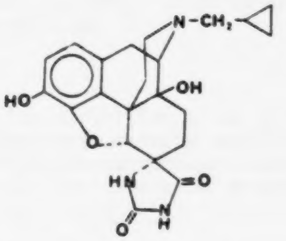
NIH 10516 Oxycodone-6-spirohydantoin

...(continued)

SUMMARY

NIH 10516 did not have opiate activity upon the mouse vas deferens preparation. Both assays' results suggested that NIH 10516 had no significant opioid activity.

NIH 10519 Naltrexone-6-spirohydantoin succinate



DISPLACEMENT OF SPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 55.2 nM in the presence of NaCl.

MOUSE VAS DEFERENS PREPARATION

	Inhibitory EC50 (M)	Maximum Response
Drug alone	2.75×10^{-6}	75.2 ± 3.63%
After naltrexone	1.69×10^{-5}	55.2 ± 8.73%
After ICI 174864	4.96×10^{-5}	76.5 ± 2.05%
After beta-funaltrexamine	4.79×10^{-6}	54.2 ± 3.75%
With equimolar concentration of sufentanil	No reversal	

SUMMARY

NIH 10519 had delta receptor agonistic activity upon the mouse vas deferens preparation. In the membrane binding assay, it was approximately 2-fold less potent than morphine.

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